FEDERAL EXPERIMENT STATION IN PUERTO RICO

of the

UNITED STATES DEPARTMENT OF AGRICULTURE

MAYAGUEZ, PUERTO RICO

REPORT OF THE FEDERAL EXPERIMENT STATION IN PUERTO RICO 1945

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FEDERAL EXPERIMENT STATION IN PUERTO RICO

MAYAGUEZ, PUERTO RICO

Administered by the Office of Experiment Stations
Agricultural Research Administration
United States Department of Agriculture

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[1] In cooperation with the Government of Puerto Rico.

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INTRODUCTION

As a result of the recent war, the role of the United States in world affairs has reached proportions far exceeding expectations. The pressing need at the outset of the war for strategic materials, such as rotenone, quinine, and rubber, left the United States no choice other than to promote the intensive cultivation of such crops in the Western Hemisphere. As a result, the interest in tropical agriculture, especially in Latin America, increased tremendously. The increased facilities for air travel and transport after the war indicate clearly that there will be further expansion of industry and agriculture to South America.

Many American servicemen who have been stationed in tropical areas during the war have shown a real interest in tropical agriculture as their future means of livelihood. Evidence of this fact is shown by the numerous inquiries from these men to the United States Department of Agriculture in Washington and to the Federal Experiment Station in Puerto Rico. This agricultural outpost maintained by the United States has 43 years of research experience with many tropical crops. In recent years, special emphasis has been placed on new crops for the Western Hemisphere Tropics, such as bamboo, *Derris*, *Cinchona*, essential oils, and spice crops. *Derris* and *Cinchona*, the sources of rotenone and quinine, have received particular attention because of their critical position in the war effort. Research during the past year on these and other crops is reported in the following pages.

PERSONNEL

Several new members were added to the station staff during the year. Norman F. Childers, formerly assistant professor of horticulture at the Ohio State University and associate in horticulture at the Ohio Agricultural Experiment Station, was appointed assistant director and plant physiologist on August 16, 1944. Other appointments included David G. White as horticulturist and plant physiologist on July 21, 1944; Edward P. Hume as horticulturist on May 11, 1945; and Caleb Pagán Carlo as scientific aide on November 16, 1944. Milton Cobin, horticulturist, was granted a year's leave of absence as of November 22 to undertake a special assignment with the Government of Puerto Rico.

The following changes took place in the personnel employed on funds provided the station by the Insular Government: The position of junior engineer on the bamboo project was vacated by Miguel Lugo López on October 20, 1944, when he left to take up graduate work in the continental United States. This position was filled November 16, 1944, by Faustino Rivera López, who resigned June 30, 1945, to accept other employment. Miss Violeta Vicente, collaborating clerk, resigned on September 30, 1944, and was replaced by Mrs. Edna E. R. de Cedó December 18, 1944.

During the absence of the director, Norman F. Childers served as acting director during various periods of the year.

Cooperation with other Government Agencies

The station received the finest cooperation from the Insular Government. The Insular Legislature appropriated the sum of $24,105 to supplement the research work at the station, particularly with tropical crops of importance to Puerto Rico, such as vanilla, spices, essential oils, and bamboo. The War Emergency Program provided funds in the amount of $104,076, which was used to employ limited technical and clerical assistance and laborers on all Federal and Insular projects.

The Insular Legislature also appropriated the sum of $23,000 for the purchase of land for the use of the station. This was in addition to the sum of $22,000 appropriated in 1944 for the same purpose. When this land is acquired, approximately 24 additional acres adjacent to the present station property will be available for expanding station plantings. The station lands are already becoming crowded because of the large number of plant accessions which have been introduced over the years. Some of the funds allotted are to be used for the purchase of a coastal strip of land in order to provide another environment typical of many areas in Puerto Rico for testing new plants and for other experiments. All of this land, like that now occupied by the station, will remain in the name of the people of Puerto Rico, but will be available to the Federal Government for its use as long as funds are provided for the maintenance of a Federal experiment station in Puerto Rico.

The War Emergency Program also provided funds for the completion of the 2,225,000-gallon water-storage tank originally started under a WPA program. This storage tank provides adequate and safe water facilities for all of the needs of the station. Funds were also provided by this agency for the drainage of a swamp area in the rear of the station buildings, including the construction of a lagoon to alleviate flooding

of the station building and lawn. Both construction projects were completed during the year.

The experiment station of the University of Puerto Rico and the Federal station continued to cooperate closely on local agricultural problems. Conferences and exchange of ideas and programs between the respective directors resulted in the coordination of all activities. The experimental work with coffee carried on by the university station was conducted at Mayaguez, and office, laboratory space, and field areas were made available by the Federal station for this purpose. The cooperative agreement between the two stations and the Soil Conservation Service was continued in the furtherance of the soil conservation research.

The College of Agriculture and Mechanic Arts of the University of Puerto Rico utilized the facilities of the station from time to time in the instruction of college students. Horticultural classes visited the station to see the experimental work in progress and to utilize for class work the large collection of tropical plants available at the station. Students were trained in laboratory procedures in the chemistry laboratories and in the plant introduction work, particularly in the maintenance and preparation of a herbarium.

The Institute of Tropical Agriculture of the University of Puerto Rico, located in Mayaguez, has cooperated with the station in various projects. The director served as a member of the board of trustees of the institute.

The extension service of the University of Puerto Rico cooperated with the station in several projects. Through the facilities of their demonstration farms, a number of experimental plantings were made. The extension service agents conducted a survey to determine the establishment of bamboo distributed by the stations, and have also given their fullest attention to other requests made by members of the station staff.

Cut and dried culms of the beetle-resistant bamboos were made available to the Puerto Rico Development Co. This agency is sponsoring the development of new industries on the island, and has initiated a project to utilize bamboo in the construction of furniture. One manufacturing shop has been established by private enterprise as a result of these activities.

The Forest Service, both Insular and Federal, continued to work closely with the station. Approximately 16 acres of land in the Guanica Insular Forest were made available to the station for plant introductions. This area is located in a dry coastal section of the island and provides another environment to test introduced plants. Other areas in the Toro Negro Unit of the Caribbean National Forest and the Maricao Insular Forest were maintained under the administration of the station for the development of *Cinchona* plantings.

The Office of Foreign Agricultural Relations sent personnel to Mayaguez for observation and training in connection with their program for the development of agricultural experiment stations in Latin America. The station has also provided considerable seed and planting material to the Office of Foreign Agricultural Relations for introduction and testing at their stations in South America.

The station has provided laboratory space, offices, and field areas for the work of the Soil Conservation Service. Close cooperation has existed between the staffs of the two agencies, and a cooperative project on grazing and forage crops was undertaken.

Office space was likewise provided for the Farm Security Administration and for the Insular plant-quarantine inspector, who is a collaborator of the Bureau of Entomology and Plant Quarantine.

The military authorities, particularly the post engineer of Borinquen Field, have been extremely helpful in providing personal services and material to the station. Through their cooperation, the station was able to install and put into operation air-conditioning equipment to be used in the study of *Cinchona*. They have, likewise, helped in many other ways, and the station has been able to cooperate with the military authorities by giving technical assistance on agricultural problems and also by providing considerable quantities of planting material for use on the military bases. The station has given several thousand feet of bamboo to the military authorities for the decoration of enlisted men's clubs and recreation centers.

The director of the station has served as a member of the USDA War Board for Puerto Rico, and the station has cooperated in the War Board's programs whenever possible.

INSECTICIDAL-CROP INVESTIGATIONS

DISTRIBUTION

By David G. White and Rufus H. Moore [1]

Derris and Lonchocarpus planting material distributed to Latin America.—The shipment of planting material of *Derris elliptica* (Wall.) Benth. and *Lonchocarpus* spp. was considerably reduced during the past year. Cooperation with the Foreign Economic Administration was confined to the shipment of 55,066 leafless stem cuttings of the Sarawak Creeping variety of *D. elliptica* to Colombia and Brazil.

In cooperation with the Foreign Economic Administration, a few small shipments of *Lonchocarpus utilis* A. C. Smith and *L. chrysophyllus* Kleinh. were made to Brazil, Guatemala, and Hawaii.

PROPAGATION

By David G. White, Merriam A. Jones, and Caleb Pagán

High-rotenone Derris clones evaluated.—During the past 4 years the 9 high-rotenone clones of the Changi No. 3 variety of *D. elliptica* introduced from Panama through the cooperation of the Goodyear Rubber Plantations Co. were shield-budded on Sarawak Creeping and St. Croix stocks. The vines which developed from the grafted buds were removed and planted as 12-inch leafless cuttings. Plants of individual clones were placed on separate terraces to maintain their identity and to provide a future source of the best material after evaluation studies are completed. The roots of the original plants of these high-rotenone clones, which had been in the field for 4 years, were harvested and analyzed by diameter classes: Small, less than 4 mm.; medium, 4 to 8 mm.; large, greater than 8 mm. There were 9 clones

[1] Now assistant professor of horticulture, University of Nebraska, Lincoln, Nebr.

harvested in 3 replicate groups and sorted into 3 sizes by root diameter; this gave 81 samples, all of which were analyzed colorimetrically for rotenone plus rotenoids. The powdered samples of small, medium, and large roots were then composited in proportion to yield to give 27 samples, which were analyzed for moisture, total chloroform extractives, rotenone, and rotenone plus rotenoids. Combining these data with the yield data, an analysis of variance was made to ascertain whether the differences among clones were significant on the bases of yield of roots, rotenone, and rotenone plus rotenoids, as well as on the bases of percentages of rotenone and of rotenone plus rotenoids. At the time of harvest some plants of the Sarawak Creeping variety grown in the same field were harvested for comparison. Although the root yield appeared normal, the Sarawak Creeping was found to be definitely lower in rotenone than similar material grown in lighter and better-drained soil. This indicated that the high-rotenone clones of Changi No. 3 may be expected to yield higher-quality roots when grown under more favorable conditions.

In yield of roots the highest-yielding clones were superior to the lowest by high significance. One clone was outstandingly high, averaging 29.7 ounces per plant, and one clone was exceptionally low, 16.6; roots of the Sarawak Creeping variety in the same field averaged 13 ounces per plant.

With regard to root quality, approximately the same order of rank was obtained whether the criterion was percentage of rotenone or of rotenone plus rotenoids. Rotenone contents varied from 6.6 to 8.0 percent with the higher values differing from the lower by high significance. Although rotenone determinations were not made for the root size groups, colorimetric determinations of rotenone plus rotenoids showed that significant differences existed; thus, for all clones it was found that small and medium diameter roots were about equal in rotenone plus rotenoids but that large roots were somewhat poorer by a highly significant difference. Ranking of the clones within root diameter groups according to root quality gave approximately the same order in all three size groups.

With regard to the yield of rotenone per plant (the rotenone percentage multiplied by the root yield), only the three highest clones were significantly better than the poorest clone. When the yield of rotenone plus rotenoids (percentage rotenone plus rotenoids times the root yield) was used, the order of ranking was similar. Since this colorimetric criterion was also applied to the root diameter groups, it could be seen that in general the large roots contained the most rotenone plus rotenoids. Medium roots were second and the small roots last. The differences were highly significant. No significant differences existed among the clones on the basis of yield of rotenone plus rotenoids in small- or in medium-diameter roots; however, in the large roots, significant and highly significant differences were apparent. Although the large roots were somewhat poorer in quality than the smaller ones, the bulk of the rotenone produced by the plant was in the large roots. Therefore, the practice sometimes recommended in the literature of discarding the large roots is not sound.

The identifying numbers of the 9 clones of high-rotenone *Derris* were previously in accordance with the numbering of the 200 plants first tested at the Goodyear Plantation in Sumatra. To simplify the designations and to avoid confusion with numbers assigned by other agencies having material from the same source, the clones have now been renumbered from 1 to 9 in descending order of rotenone percentage as found in the foregoing evaluation experiment. The original Goodyear numbers in Sumatra and the corresponding new MG (Mayaguez-Goodyear) numbers are as follows:

Old Sumatran Number	New MG Number	Old Sumatran Number	New MG Number
122	1	76	6
10	2	33	7
31	3	73	8
107	4	104	9
23	5		

Based on the new MG numbers, the first evaluation experiment gave the following order: 1, 5, 6, 2, 4, 7, 9, 8, 3. The correlation coefficient, 0.52, between the rotenone percentages obtained in 1942 and the recent values was not significant. The source of poorest agreement was clone 3, which came last in the previous alinement. If clone 3 is omitted, the correlation coefficient, 0.768, is significant but not highly significant.

Type of cutting had little effect on root yield or rotenone content.—In the 1944 annual report, data were given on the development of rotenone and rotenoids in the Sarawak Creeping variety of *Derris elliptica* started from two types and three sizes of cutting material. Data were given for the harvests made at the ages of 24 days and 2, 3, and 8 months. During the past year harvests were made at 14 and 20 months after propagation. At 14 months, the yields and analyses of roots showed that, after the growth made during the rainy season, there was more variation among replicates of the same type of cutting than among the different types of cuttings. At 20 months the plots started from hormone-treated leafy cuttings yielded the most roots, 875 pounds per acre, followed by untreated leafy, medium leafless, and small leafless at about 740 pounds per acre. The large leafless series yielded the least, 660 pounds per acre. However, the variation among replicates was great. Likewise, rotenone percentages varied from 2.7 to 6.3, with no treatment being particularly superior. Although the root yield increased by as much as several hundred percent during the last 6 months of growth, the percentage of rotenone, a little below the standard for this variety, remained about the same. However, regardless of the type of cutting used, more or less normal plants developed and early differences tended to disappear as maturity was approached.

DERRIS CUTTING EXPERIMENTS

By David G. White and Aida G. Villafañe

The preparation and planting of derris cuttings.—Two-node cuttings of the Sarawak Creeping variety of *Derris elliptica* were cut 1 cm. above the distal bud and at various distances below the basal bud. After 42 days large differences were found in numbers and weights of roots in

relation to the location of the basal cut. It was concluded that the basal cut should best be made at least 3 cm. below the bud. Observations made during the experiment indicated that the basal bud might be associated with the location of roots. Such a phenomenon would imply the presence of plant-growth factors such as auxins, which might be transported from the bud through bark tissues. However, a series of experiments in which cuttings were notched at various distances above and below the basal bud had no apparent effect on subsequent rooting.

Cuttings placed at an angle of 45° from horizontal produced a large number and a greater weight of roots in 46 days when the distal bud faced up rather than down. The layering of derris cuttings at angles of 15°, 30°, 60°, and 90° from horizontal showed no consistent differences in rooting and was therefore not important in *Derris* propagation.

Measurements were made of the early growth of derris roots.—V-shaped glass-sided boxes with removable shades permitted the observation and daily measurement of individual derris roots. Three 12-inch pencil-size cuttings of three varieties were planted in a mixture of one-half sand and one-half clay loam. The first roots appeared at the glass in 13 days in all varieties. A gummed label adhered to the glass marked the origin of appearance of each root and also carried the individual root number. Linear measurements were made every 24 hours for 30 days. Most roots attained a diameter of about 1 mm., and many produced laterals. Some individual roots elongated as much as 1.6 cm. per day, although the average daily rate was 0.47 cm. Roots of Changi No. 3 (Rio Piedras clone) elongated at an average daily rate of 0.38 cm., Sarawak Creeping 0.45 cm., and St. Croix 0.52 cm. The rate of elongation usually decreased with time for any individual root and often ceased entirely, although such roots remained apparently healthy. Roots growing through a soil cavity were observed to elongate more rapidly than when in the soil mixture. In this study the St. Croix variety had the largest total increment of root elongation at the end of 30 days, not only because these roots had the largest daily rate of elongation but also because more roots were apparent at the glass.

Leaf measurements indicated that top growth and root growth were more or less opposing in this experiment. Thus, the roots elongated most rapidly during a period of little leaf growth and vice versa. Three of the glass-sided boxes filled with one-half sand and one-half clay loam mixture were planted with three cuttings each of clone 6 of Changi No. 3. One box contained cuttings of large diameter (1.9 to 2.2 cm.), a second box of medium diameter (1.2 to 1.4 cm.), and a third box of small diameter (0.6 to 0.8 cm.). Measurements were begun 12 days later, and observations continued for 30 days. The 3 large-diameter cuttings produced a total of 35 roots at the glass, the medium cuttings 18 roots, and the small cuttings 14 roots. Roots from the larger-diameter cuttings had an average rate of daily elongation per growing root of 0.82 cm., medium cuttings 0.70 cm., and small cuttings 0.68 cm. In general the rate of root elongation became less with time, and there was no measurable root development during the last 6 days of the 30-day observational period. However, top growth meanwhile increased rapidly and partly accounted for the lack of root development at this time.

Protoxylem points not correlated with root growth or rotenone content.—The roots of the several varieties of *Derris* previously reported in the elongation study were excavated for anatomical study. The technique employed to observe the number of protoxylem points consisted of making fresh cross sections with a straight-edge razor, washing in water, and momentarily staining with safranine, which emphasizes lignified tissue such as the protoxylem cells. Roots were classified as having 4, 5, 6, or 7 protoxylem points.

Considerable variation occurred in the rate of root elongation within any one class of protoxylem points. Thus, the conclusion was reached that in this experiment no correlation existed between the rate of elongation and the number of protoxylem points in the roots.

Worsley and Nutman (*30*)[2] found that longitudinal bands of cells in the secondary cortex of derris roots are the first to contain rotenone and that these bands are invariably arranged opposite to the protoxylem strands in corresponding number. Later rotenone deposition occurs in scattered cells of the xylem but particularly near the primary medullary rays, which also correspond in number to the protoxylem points. This information suggested that the rotenone content of mature roots might be correlated with the number of protoxylem points.

A 3-year old plant of Changi No. 3 clone 5 which had shown an average of 9.3 percent of rotenone in a root sample taken in 1942 was chosen for this study. The entire root system was removed, and nine comparable roots of pencil-size diameter were chosen for study. Four of these roots had four protoxylem points, three had five points, and two had six points. The longer roots were divided into distal, middle, and basal sections for chemical analyses.[3] The percentage of rotenone plus rotenoids was determined colorimetrically for these root samples. The data showed as much variation within a single root as among roots, and no correlation could be seen between quality and the number of protoxylem points.

The probability that the number of protoxylem points might differ in different varieties of *Derris elliptica* was deemed of interest. Ten pencil-sized comparable cuttings of each of the following varieties were rooted in sand: Changi No. 3 (Rio Piedras clone), high-rotenone Changi No. 3 clone MG 8, Sarawak Creeping, and St. Croix. After roots had developed, the cuttings were lifted and all roots sufficiently large were sectioned for anatomical study, as described previously. A total of 94 roots were observed to have 4, 5, 6, 7, or 8 protoxylem points. Statistical analysis showed that Changi No. 3 roots of the Rio Piedras clone had a smaller number of protoxylem points to a highly significant degree than any of the other 3 varieties. Sarawak Creeping roots were found to have the largest number of protoxylem points to a highly significant degree. St. Croix and Changi No. 3 clone MG 8 roots had average numbers of protoxylem points for the 4 varieties studied. The frequency of different numbers of protoxylem points can be considered to be a varietal characteristic.

Mature plants of the St. Croix variety of *Derris* were dug relatively intact. The older roots near the region of the original cutting were separated into 3 classes according to diameter: 0.1 to 0.3 cm., 0.8 to 0.9

[2] Italic numbers in parentheses refer to Literature Cited, p. 61.

[3] Chemical analyses by Merriam A. Jones and Caleb Pagán.

cm., and 1.5 to 2.2 cm. In 40 observations there were no consistent differences found in number of protoxylem points of roots in the 3 classes. It was noticed, however, that only 4 or 5 points were found in these roots, as compared with observations of young roots arising from fresh cuttings in which 6 and 7 points were not unusual. The reason for the absence of 6 and 7 points in the older St. Croix roots is not known.

DERRIS GRAFTING EXPERIMENT

By Merriam A. Jones and William C. Cooper[4]

Grafting of Derris varieties did not change characteristic rotenone content of roots.—The several strains of *D. elliptica* growing at the station are characterized by different yields of root and rotenone content. The St. Croix variety is high in yield of root and low in rotenone; the Sarawak Creeping variety gives a medium yield of root of medium rotenone content, and the Changi No. 3 MG clones introduced from Panama are characterized by medium yield of root high in rotenone. An experiment was carried out to determine whether the superior qualities of these strains could be combined by grafting. High-rotenone Changi No. 3 clone MG 1 was grafted with St. Croix material, and in a smaller parallel experiment Sarawak Creeping was grafted with St. Croix. In one set of grafts Changi No. 3 and St. Croix material were used as both stock and scion, being whip-grafted one on the other and upon themselves. Including the controls in which no grafts were made, there were 6 treatments of 10 plants each. At the age of 11 months the plants were harvested and the roots divided into two diameter groups, those smaller than 5 mm. in diameter and those 5 mm. and over. To obtain a measure of quality of the separate small samples, the roots were analyzed for percentage of rotenone plus rotenoids. Suitable composites were then made for additional colorimetric tests (*13*) and complete rotenone analysis (*5, pp. 64-66*).

Chemical analyses of the roots showed that the effect of scion on stock was negligible. The most striking feature in the data was the wide variation among plants of the same clone, especially with regard to quality as measured by the rotenone plus rotenoids or the rotenone content. No correlation could be found between quality and yield in any of the treatments.

With regard to the ratio of rotenone to rotenone plus rotenoids, the St. Croix variety root tended to have a low ratio of about 0.35 to 0.45, whereas the higher-quality Changi No. 3 and Sarawak Creeping varieties had a ratio of 0.50 to 0.55.

DERRIS VARIETAL EXPERIMENT

By Merriam A. Jones, Rufus H. Moore, and Caleb Pagán

A comparison of three Derris varieties completed.—An experiment was completed comparing three varieties of *D. elliptica:* Sarawak Creeping, Changi No. 3 (Rio Piedras clone), and St. Croix. The Sarawak Creeping and Changi No. 3 varieties were introduced in 1931 by the Agricultural Experiment Station of the University of Puerto Rico at Rio Piedras and subsequently transferred to this station. The agricultural experiment station in St. Croix, U. S. Virgin Islands, grew

[4] Formerly of the Office of Foreign Agricultural Relations.

three plants of *D. elliptica* from seed introduced from Java in 1932. Cuttings from these three plants were planted at Mayaguez in 1935 and are referred to as the St. Croix variety. The St. Croix variety produced 2,948 pounds of dry root per acre, which was greater by high significance than the yield of Changi No. 3, 1,264, which in turn was significantly greater than the yield of Sarawak Creeping, 1,028. As to root quality as measured by the rotenone content (*5, pp. 64–66*), the Sarawak Creeping variety led with a mean rotenone content of 5.5 percent, dry basis; this was greater by high significance than the rotenone content of the root of Changi No. 3, 4.1 percent. The root of the St. Croix variety was poorest, 2.0 percent.

Under the conditions of this experiment, the St. Croix variety produced by far the most root and the Sarawak Creeping by far the best-quality root. To be sure, neither of these criteria is decisive; insecticidal value per acre should be a consideration. However, from the producer's standpoint this is perhaps not so important as rotenone per acre. Although it is generally stipulated that the ratio of total extractives to rotenone be at least three, roots are purchased principally on the basis of rotenone content. Therefore, the product of dry root and rotenone content was calculated for these three varieties with the following results: St. Croix, 58.6; Sarawak Creeping, 55.6; and Changi No. 3, 51.0 pounds of rotenone per acre. The differences between St. Croix and Sarawak Creeping and between Sarawak Creeping and Changi No. 3 were not significant, but the difference between St. Croix and Changi No. 3 was almost highly significant.

This experiment showed that the Changi No. 3 variety (Rio Piedras clone) was inferior to the St. Croix and Sarawak Creeping varieties. The two latter have certain advantages: With the St. Croix variety there are more roots to be harvested per acre. This may be an advantage if a mechanical harvester were used because the roots would be stronger and less easily lost in the soil. For the St. Croix variety it may also be said that the plants with their characteristically large root systems are extremely vigorous and sturdy; there may be more basis for hope to raise the rotenone level of this low-rotenone root than to increase further the rotenone content of the richer, less vigorous Sarawak Creeping. However, a serious disadvantage of the St. Croix root was that the rotenone content was so low as to be unacceptable in the prewar derris market. For the Sarawak Creeping variety it may be said that the smaller quantity of high-quality root is cheaper to handle in the drying and grinding process and the rotenone content was sufficiently high for the market.

In five of the Sarawak Creeping plots and in all of the Changi No. 3 plots, 5 of the 10 rows were planted with large cuttings and the remainder with small. The subplots were harvested to obtain the root yield and rotenone-plus-rotenoids content. In root yield and in content of rotenone plus rotenoids, no statistically significant differences ascribable to size of cutting were found.

Row plots statistically more efficient than square or rectangular plots.—In conjunction with the harvesting in this *Derris* variety experiment, data were obtained to determine the minimum size of plots that could be utilized in this type of experiment and still yield significant results.

Plots were harvested by fractions of rows, each row of 14 plants being taken as two 5-plant samples and one 4-plant sample. Yields of roots were taken and measurements made of rotenone plus rotenoids from which rotenone percentages were calculated by means of the ratio of rotenone to rotenone plus rotenoids found for the 30 large plots. The St. Croix variety was not considered in this study. The results are tabulated in table 1 for the number of replicates necessary to show significant differences in yield of root, percentage of rotenone, and yield of rotenone (*19, p. 65*).

TABLE 1.—*Minimum number of replicates necessary to demonstrate significant treatment differences at the 5-percent level in Sarawak Creeping and Changi No. 3 varieties of Derris elliptica*

Plot shape and size	Minimum replicates to show differences in—		
	Yield of root	Rotenone content [1]	Rotenone per unit area
	Number	*Number*	*Number*
Block, 8 plants x 14 plants (10 plots)	4	3	18
8 x 14 (4 plots)	3	3	11
5 x 5	3	3	12
4 x 4	8	3	9
2 x 4	8	3	11
Row, 1 x 14	4	3	7
1 x 10	4	3	12
1 x 5	6	3	24
1 x 4	11	3	16

[1]Calculated from rotenone plus rotenoids except for 8 x 14 plots in which rotenone was measured as such in composite samples.

The data show that the variability was great enough to require a considerable number of replicates in some cases. Apparently differences in rotenone content of the magnitude 3.9 to 5.6 as in these two varieties can be demonstrated with comparatively few replicates. In most cases more replicates were needed to demonstrate significance of differences in yield of root or yield of rotenone. In general row plots were more efficient than block plots, square or rectangular.

Obviously, the value of the information obtained from these data is only qualitative; it can be used only in a rather general way in designing experiments. For example, economy of material can be achieved using small row plots with sufficient replications instead of large block plots. Besides saving material and labor, soil and plant heterogeneity can be minimized. It may be tentatively concluded that in this type of experiment valid results could be obtained by using about 8 replicates consisting of 10-plant row plots.

DERRIS MULCHING EXPERIMENT

By David G. White

Mulching Derris appeared favorable.—Several experiments were started on the use of mulch in the growing of *Derris*. Cane leaves, decomposed stems and leaves from harvested *Derris*, and lemon grass leaves were used. The leaf mulches considerably reduced the time required for weeding; 188-plant plots with 6 inches of cane leaf mulch required 9 hours 42 minutes to clean; those with 12 inches of mulch required 6 hours 4 minutes; whereas those not mulched required 25

hours 20 minutes. The time necessary to remove nut grass from the unmulched plots was the chief reason for the differences. The *Derris* debris in the form of decomposed leaves and stems apparently contained additional weed seed and was an excellent medium for their development. From a practical standpoint the *Derris* debris has not proved to be a good mulch. Cane leaves formed a tighter mulch than lemon grass leaves, although the latter were more easily handled.

In one of these mulching trials the temperatures 6 inches beneath the soil surface were continuously recorded with a Foxboro Soil Thermograph having two sensitive units. One unit was buried in soil covered with 12 inches of lemon grass mulch, and the other unit was buried in soil of an unmulched plot. The lowest recorded temperature over a period of 28 days was 73° F. between 7 and 9 a. m. in both plots. In general, the lowest soil temperatures always occurred during these same morning hours and were about equal for both plots. The highest recorded temperature for soil of the mulched plot was 82° between 3 and 6 p.m. and 97° between 4 and 6 p.m. in the unmulched plot. Soil temperatures in the unmulched plot, in particular, usually began to rise between 8 and 9 a.m. The largest difference in temperature between mulched and unmulched plots was 16°, which occurred at 4:00 p.m. on a relatively hot day. Air temperatures ranged from 66° between 7 and 8 a.m. to 94° between 2 and 3 p.m. The differences in soil temperatures between the mulched plots and those not mulched may have considerable influence on the development of the roots and rotenone deposition.

Differences in soil moisture were particularly evident during the dry season. Soil samples taken at a depth of 6 and 12 inches were found to be considerably higher in moisture in the mulched plots. These differences continued during the rainy season but were not so great.

Derris floral development associated with viability of pollen.—Over 50 percent of the pollen taken from MG clones of Changi No. 3 flowers which lacked about 1 day of being open germinated after 17 hours on 5-percent sucrose and on 5-percent dextrose. On the other hand, no germination occurred with pollen taken from flowers which opened the same day. Therefore, best results in hand pollination would be expected with pollen taken from flowers not yet open. These observations also may explain the reason for the scarcity of derris seeds under our conditions; i.e., staminate and pistillate organs probably mature at different times.

DERRIS PHYSIOLOGY

By Rufus H. Moore

Derris produced higher quality roots in the field than in pot cultures.—Grown in the open field *D. elliptica* has always produced roots higher in quality than when grown in pots in the greenhouse. Ten trellised 27-month-old plants of a clone of the St. Croix variety were harvested from a field of Catalina clay, and their roots were sorted according to reaction to the Durham test (*24*). On a dry basis, the better or strongly positive roots averaged 3.8 percent of rotenone and the poorer or moderately positive roots averaged 2.1 percent. Progeny of these plants were used in a greenhouse experiment to determine the effects of high- and low-

carbohydrate levels of nutrition on rotenone content (*17*). The results showed that low-carbohydrate plants, which grew rapidly, yielded roots averaging 2.4 percent of rotenone, and high-carbohydrate plants, which grew slowly, yielded roots averaging only 0.5 percent of rotenone. This indicated that rate of growth and percentage of rotenone were related.

On the basis of carbohydrate analyses the four series were divided into two distinct classes, the field-grown roots being notably lower in sugars and starch than the pot-grown roots. A point of special significance is that even the rapidly growing potted plants had greater percentages of carbohydrates than either of the outdoor series. An inverse relationship appeared to exist between the percentages of carbohydrates and of rotenone in the four series.

In the field the rate of root growth of both the St. Croix and the Changi No. 3 varieties was unchecked, but in jars the rate of root growth declined when the limited substrate became ramified by the root systems. This difference in the growth rate of field- and pot-grown plants affected both the diameters of the roots and their rotenone contents. With the outdoor plants the roots were not only thicker but also of a higher rotenone content than similar roots grown in pots.

Some of the differences in root diameter reported were associated with the media in which the plants grew. In general, the proliferation of fine roots was suppressed by clay and favored by sand or enriched loam. An increase in the number of fine roots involved competition among many root tips and resulted in a slower average rate of elongation. The abundance of fine roots, absence of large roots, pronounced accumulation of carbohydrates, and lowering of rotenone content were evidence that pot culture over a period of time induced a high-carbohydrate level of nutrition in *Derris*. Several factors contributed to the tendency of pot-grown *Derris* to become high in carbohydrates. The effect of friable media on the production of many competing, fine roots has already been mentioned. The growth rate was further checked when the roots became pot-bound. Even though the roots became pot-bound, the growing shoot required progressively more water. In sand cultures, especially, the demands of transpiration required two liberal applications of nutrient solution daily. Diurnal fluctuations in the water supply may have been sufficiently sharp to have checked growth to some extent, even though the plants seldom reached the stage of incipient wilting. Limitations of the data do not permit the conclusion that all of the reduction in root quality of pot-grown *Derris* can be ascribed to an altered nutritional level. The results did show, however, that a high-carbohydrate level of nutrition was one of the important factors involved.

Pruning and defoliation of derris plants reduced carbohydrates but not rotenone.—When derris plants growing in the field were cut back to stumps and new leaves removed regularly until the plants were almost dead, carbohydrates were almost depleted in the roots although the concentration of rotenone remained unaltered (*17*). The results of this field test were confirmed in the following greenhouse experiment. Well-rooted cuttings of the Changi No. 3 variety were grown in 5-gallon glazed jars filled with quartz sand. The plants were given a complete nutrient solution applied frequently so that the sand was thoroughly

moist but well drained. The cultures were flushed periodically, and the vines were supported by unshaded trellises. Four series were started on July 15, 1941. Series 1, the initial control, was harvested in early January 1943, when the vines of series 2 and 3 were cut back to short stumps. Leaves and vigorous new shoots were removed from series 2 and 3 at 4-day intervals. Series 2 was harvested in early April 1943, when the carbohydrate reserves in roots had been appreciably depleted, and series 3 in late July 1943, when the plants were nearly or partly dead. Series 4, the final control, was allowed to grow without pruning or defoliation and was harvested simultaneously with series 3. Each series included two replicates of nine plants so distributed in the greenhouse that any variations in growing conditions would be equalized.

The carbohydrate reserves were definitely and progressively reduced in the plants of series 2 and 3. Leaf removal had less effect on other plant constituents. As the reduction in carbohydrates became more acute, a consistently progressive increase in the concentrations of total extractives, rotenone, and rotenone plus rotenoids occurred in the fine roots, but not in roots of greater diameter. Under greenhouse conditions removal of the leaves reduced carbohydrate reserves more drastically than in the field, but under either set of conditions leaf removal did not affect materially the concentration of rotenone in the fresh roots.

The fact that once rotenone is formed in roots on the derris plant it is not digested, translocated, and reutilized like carbohydrates, fats, and organic-nitrogen compounds has a direct bearing on both experimental procedures and commercial practices. Any experiment designed to test the effects of a given set of conditions on rotenone should allow for the rotenone present in the roots at the beginning of such a study, should provide for careful control of the growth rate of the plants, and should ordinarily insure the simultaneous harvest of tops and roots. Applied to commercial practice, the starvation studies mean that rotenone is not lost as a result of seasonal changes or agronomic practices, and that any appreciable interval between removal of tops and digging of roots tends to decrease the amount of carbohydrates present and thereby increase the rotenone percentage, although the actual rotenone content remains the same.

Quality of derris root lowered with increasing altitude.—The effect of altitude on the quality of derris root was noted in test plantings of trailing plants harvested in Puerto Rico in 1942 (*16, p. 12*) and 1944 and in roots gathered from trellised plants by the writer in Guatemala in 1943. The root samples were dried and analyzed for total extractives, rotenone, total sugars, and starch. The sum of the percentages of total sugars and starch is reported as reserve carbohydrates.

Both total extractives and rotenone determine the quality of derris root. Since variations in these two constituents followed similar trends, as shown in table 2, rotenone can be used as an index to the quality of these root samples.

TABLE 2.—*Analysis on a dry-matter basis of derris roots from several altitudes in Guatemala and Puerto Rico* [1]

Locality and year of harvest	Site	Elevation above sea level	Mean annual rainfall [2]	Age at harvest	Total chloroform extractives	Rotenone	Carbohydrates
		Feet	*Inches*	*Months*	*Percent*	*Percent*	*Percent*
Guatemala, 1943	Guatemala City	5,000	43.1	33	6.7	2.0	37.1
	Finca Montevideo	3,650	204.1	14	11.2	3.5	----------
	Finca María Santísima	2,050	99.0	24	16.1	6.3	24.2
	Finca El Baúl	1,700	----------	24	14.6	5.3	26.5
	Finca Velázquez	910	----------	25	14.7	5.5	33.2
Puerto Rico, 1942	Maricao	2,400	98.9	27	6.2	2.0	27.4
	Cidra	1,400	79.1	24	13.3	4.3	19.0
	Utuado	380	73.4	28	19.6	6.0	----------
	Sabana Grande	220	49.1	24	16.4	4.5	10.9
	Vega Baja	215	70.2	28	20.7	6.0	5.9
	Mayaguez	50	76.3	27	21.3	6.8	13.1
uerto Rico, 1944	Orocovis	1,970	68.9	19	5.5	1.9	37.8
	Cidra	1,410	89.7	19	10.4	3.9	28.3
	Humacao	100	91.2	19	17.4	6.2	20.4

[1] Analyses for total chloroform extractives and rotenone by M. A. Jones; analyses for carbohydrates by M. A. Jones and Gilda C. Vicente.

[2] Mean annual rainfall during period of growth.

The data show that several factors influenced rotenone content. At higher elevations the effect of differences in rainfall on root quality was masked by the influence of altitude, as shown most clearly in the data from Puerto Rico. *Derris* grown at Maricao under 98.9 inches of rainfall yielded roots of practically the same quality as *Derris* at Orocovis under 68.9 inches of precipitation.

Some factor specific to altitude was important as indicated by the inverse relationship between rotenone and altitude. That such a factor could have been temperature is suggested by the increase in temperature that accompanies a decrease in altitude. For example in Puerto Rico, the mean annual temperature at 3,000 feet is about 67.6° F., at 2,075 feet about 71°, at 1,400 feet about 73°, and at approximately sea level about 77.5°.

Latitude and land-mass effects give weight to the possible role of temperature, because at similar elevations derris root was of higher quality in Guatemala than in Puerto Rico. In Guatemala *Derris* was growing at about 14°40′ north latitude, approximately 247 miles closer to the Equator than the *Derris* in Puerto Rico at 18°15′ north latitude. Since the results in both Guatemala and Puerto Rico show pronounced effects of altitude on rotenone content and because temperature rises as altitude and latitude decrease, it appears probable that higher altitudes would be required to lower root quality in Guatemala than in Puerto Rico. Also it should be recalled that the Pacific watershed of Guatemala is part of a relatively large land mass on which temperatures are not moderated to the extent that they are in Puerto Rico, a small land mass directly in the path of trade winds.

Varietal selection and soils might have accentuated the observed differences in the quality of Guatemalan as compared with Puerto Rican roots. Although the plants in both places had the characteristics of the Sarawak Creeping variety, they were sufficiently different to be distinct. The exceptionally friable volcanic soils of Guatemala have the texture of fine sandy loams, which are supposed to be more favorable to quality

in derris root than the well-drained friable clay or clay loam soils of Puerto Rico.

On the other hand, the cultural method employed in Guatemala would tend to decrease observed differences in quality. Guatemalan *Derris* was trained on poles, but Puerto Rican *Derris* was allowed to trail on the ground. Since trellising lowers the percentage of rotenone (*26, pp. 38–40*), derris roots secured in Guatemala would tend to have less rotenone.

The influence of altitude on root quality apparently disappeared at higher elevations in Guatemala than in Puerto Rico. Therefore, in terms of plant response, altitudes as high as 2,000 feet in Guatemala may be considered ecologically equivalent to much lower altitudes in Puerto Rico. It was at lower altitudes, in the ecological sense, that the dominant influence of altitude receded and variations in water supply could exert their normal influence on root quality.

Mold development caused loss of derris cuttings in transit.—Delays in shipment of cuttings of *Derris elliptica* to Latin America often increased the loss caused by molds. Bordeaux mixture, sulfur dust, and lime, used alone and in combinations, were tested for preventing mold development. After treatment the cuttings were wrapped in the usual manner for shipment and stored in a ventilated shed for a period of 15 days.

The effect of the treatments on mold development could not be determined when the bundles were unwrapped, because of similarity in the appearance of mold spores and the dusts used to treat the stems. All treatments caused noticeable shriveling of the stems; those that included lime had produced a definite patchy blackening of the bark, but sulfur dust caused no such damage.

The cuttings were planted in unshaded field nurseries, and records of establishment were taken at the end of 3 months. Based on survival of the controls, 50 percent of the lime-treated cuttings became established plants, 51 percent of the 1 : 10 bordeaux-lime-treated, 53 percent of the 1 : 5 bordeaux-lime-treated, and 66 percent of the surfur-treated. The drying action of dusts adhering to the cuttings appeared to have been the principal factor which reduced the percentage of survival.

In another experiment, methods of applying the sphagnum moss used to supply moisture during transit were studied. The moss was thoroughly wetted, the excess water squeezed out, and the compressed cake loosened to a fluffy mass by whirling it in a rotating box. Two methods of applying the moss to bundles were compared: (1) Two bundles were prepared by packing moss into the larger cracks between the stems and then covering the bundle with additional moss as the wrapping in oiled paper proceeded. It was difficult to secure uniform coverage with peat moss by this method. (2) Damp moss was spread from ½ to ¾ inch deep in a rectangle at the end of the oiled paper, and both moss and paper were rolled together around the cuttings. With the paper under continuous tension until the first turn was finished, the moss was compressed into a uniform, self-felting layer entirely covering the stems. The bundles of stems were weighed before the moss was applied, and again 15 days later after the moss was removed. The losses in stem weight for the two methods were 0.5 and 2.7 percent, respectively. Weights taken at the beginning and end of the storage period showed that each package

lost 6.5 percent of its original weight. These results showed that most of the decrease in weight during storage was due to water lost from the sphagnum. The first method of packing required 223 grams of air-dry moss per bundle, whereas the second method required only 121 grams and took much less time.

CHEMICAL INVESTIGATIONS

By Merriam A. Jones and Caleb Pagán

Effect of storage on undried derris root studied.—In connection with the drying of derris root it was previously shown (*12, pp. 18–19*) that fairly rapid drying in the sun or in the shade resulted in no loss of toxic constituents but that, in some trials, when undried root was stored, a considerable decrease in toxic value took place (*14, pp. 10–13*). It was possible to store fresh root without deterioration, but under some conditions it underwent as much as 72-percent loss of rotenone in 3 months. Deterioration was prevented more or less by steaming or bromine treatment or both. This indicated that deterioration during high-humidity storage was brought about by an enzyme system which was inactivated by the steaming or bromine treatments. It was also noted that the degree of deterioration was proportional to the amount of browning of the plant tissue that occurred.

In further experiments the procedure except for the storage conditions was similar throughout. Fresh root 3 to 10 mm. in diameter of the Sarawak Creeping variety of *Derris elliptica* was harvested, washed, and cut to 2-cm. pieces. After mixing, samples were taken for storage in closed vessels and for sun and shade drying. The moisture content of the whole lot, 68.1 percent, was determined in quadruplicate by heating in the oven at 105° C. alternated with desiccation over $CaCl_2$ until minimum weight was attained. After the prescribed treatment the samples were dried at 80° for ½ hour and at 50° for several 4-hour periods until air-dry, 6 to 8 percent moisture. At this time the pieces were cut with pruning shears and duplicate 1-gram samples taken for moisture determination. The remainder was then ground through the 2-mm. and 0.5-mm. sieves of a Wiley mill, after which determinations were made of moisture content, total chloroform extractives, rotenone, and rotenone plus rotenoids. From the data, including the original and final weights, the losses of dry matter, extractives, rotenone, and rotenone plus rotenoids were calculated.

Duplicate samples were submitted to the following treatments: (1) Control, dried immediately; (2) autoclaved 10 minutes at 120° and dried immediately; (3) stored 3 months over water; (4) stored over water (500 ml.) containing bromine (1 ml.); (5) stored over water in a chamber which was repeatedly deaerated and flushed with carbon dioxide; (6) autoclaved, kept sterile and stored over water; (7) autoclaved, exposed to air and stored over water; and (8) allowed to dry slowly in the laboratory under poor drying conditions. Losses of extractives, rotenone, and rotenone plus rotenoids were small and erratic. Thus far the results in the several storage experiments undertaken have been inconsistent; rotenone losses have varied from 0 to 72 percent. As shown in the next section, the results apparently depend directly on storage conditions.

Storage loss of rotenone ascribed to root formation and oxidation.—In previous storage experiments with fresh derris root it was found that rotenone losses were due, at least in part, to oxidative enzyme action and that storage conditions favoring rooting likewise favored rotenone loss. It was also found that storage of root in loosely stoppered jars resulted in the formation of many new roots on the pieces but storage in sealed jars resulted in no new roots. Therefore, this technique was available as a method to determine the contribution of each of these factors, rooting conditions and oxidation, to the loss of rotenone during storage.

For this purpose sixteen 100-gram samples were stored, eight airtight and eight loosely stoppered but sufficiently closed to avoid drying. Duplicates of each type of storage were removed at intervals for analysis. After 2 weeks' storage, samples were taken and it was observed that new roots had formed in all loosely stoppered jars except one to which 1 ml. of toluene had been added at the time of storage. Mold had developed on all of these samples. On the samples removed, roots 5 to 15 mm. in length had formed on 20 percent of the pieces stored with loose stoppers, as compared with only 2 percent in sealed jars. Analysis showed about 10-percent loss of dry matter in both treatments, but practically no change had occurred in total extractives, rotenone, or rotenone plus rotenoids.

After storage for 3 weeks another set of four samples was taken. Those pieces stored unsealed had roots from 8 to 30 mm. long on about 30 percent of the pieces. One of these was sorted into two samples for analysis, pieces with roots and pieces without roots. In this way it was found that, although the pieces were in the same jar, the one with roots showed over 20-percent loss of rotenone, as compared with a negligible loss in those without roots. The duplicate sample of the above was not separated into rooted and unrooted pieces, and the rotenone loss in this sample was found to be about equal to the calculated total loss in the sample in which the pieces were separated. Considerable mold was present on one of the sealed samples, but neither showed rotenone loss.

After 5 weeks' storage another set of samples was taken, and the sealed ones were divided into rooted and unrooted samples. From 20 to 30 percent of the pieces had new roots up to 25 mm. long, which had withered during storage. The part of the sample having new roots showed about 30-percent loss of rotenone, as compared with about 10-percent loss for the unrooted. Some of the root was punklike from age and some mold was present, but neither of these properties appeared to be related to rotenone losses. The sealed samples showed only small rotenone losses.

After storage for 3 months, the last four samples were taken. To one of the unsealed jars 1 ml. of toluene had been added, and this served to prevent root formation. The other unsealed sample had roots and was separated into two samples. The parts showed about 66-percent rotenone loss in rooted pieces and about 50-percent loss in unrooted. The unsealed sample in which toluene had prevented root formation also showed about 50-percent loss of rotenone. The sealed samples, one with toluene and one without, showed only small rotenone losses.

From these results the following conclusion was drawn: The presence of mold or of punklike appearance in stored undried derris root

was not necessarily accompanied by loss of rotenone and other toxic constituents. The main factors that result in deterioration with respect to rotenone and other toxic constituents appear to be: (1) A loss of rotenone accompanied by root formation. This process occurs during the first month and is followed by (2) a deterioration due to oxidation. Since both of these factors are effective only in living root, deterioration can best be prevented by rapid drying.

Sun drying resulted in no rotenone loss.—In another part of this experiment, duplicate 200-gram samples were dried as follows: (1) Control; (2) vacuum oven over $CaCl_2$; (3) whole pieces, sun-dried; (4) whole pieces, shade-dried; (5) pieces split to 2-mm. lathes, sun-dried; (6) split pieces, shade-dried. The splitting served to check whether increasing the surface exposed would result in an appreciable loss of rotenone. Sun drying of whole pieces required about 5 days, but in the shade the drying was much slower and required about 15 days. With split pieces sun drying was complete in 2 days and shade drying in 4 days. Of course, these rates depend to a great extent upon the weather conditions, especially humidity, and also upon the presence of air currents. In these samples no losses of dry matter, extractives, rotenone, or rotenone plus rotenoids could be demonstrated. Therefore, any convenient method for drying can be used.

If harvests are to be made during the wet season, sun drying is only practicable if provision is made to protect the roots from rain. Artificial drying may under certain conditions be feasible; a building could be fitted with shelves and warm air circulated. Likewise, a tunnel drier could be used through which the roots could be passed countercurrent to an air current, with or without additional heat. A simple drying procedure would be on wire, fence, or bamboo shelves about 3 feet apart vertically, with their long axis in a north-south direction. The upper shelf could be roofed with thatch or glass. This would allow for east and west sunning of the roots and protection from rain. The drying area should be located in a valley or a cut where breezes are always present. Cool air coming down a cut is generally somewhat lower in relative humidity than warm stagnant air around the drying area. The roots could be piled on the shelves whole or cut to some extent and turned every day or so to facilitate drying.

Individual derris roots vary widely in rotenone plus rotenoids.—Previously it was shown that with large samples of derris root the maximum rotenone content occurred in roots of about 5 mm. in diameter; large variations in rotenone among roots of the same diameter were correlated with tissue patterns (*26, pp. 48–49*). The magnitude of differences in the rotenone-plus-rotenoid values of individual roots of *Derris elliptica* var. Sarawak Creeping in relation to diameter of the root and its position in the root system was determined. Two plants 46 months old and one plant 33 months old were excavated, and the position and depth of their roots noted. After drying, each root system was divided into 15 to 24 pieces, and records were made of the diameter of each piece and its position in the root system. The colorimetric test for rotenone plus rotenoids applied to each piece did not reveal any consistent relationship between this value and diameter or position. On one plant a rather gnarled root showed a low colorimetric value,

about one-third to one-sixth that of other roots of corresponding diameter. Among roots of a given diameter some were about equal but others showed variations up to about 100 percent. In general, the fine roots in the neighborhood of 1 mm. in diameter were inferior. Neither shallow nor deep roots were consistently rich or poor in quality. No diameter was found to be optimum nor did the degree of branching appear to affect quality.

CHEMICAL EXAMINATION OF MAMEY SEED

By Merriam A. Jones and Harold K. Plank

Mamey seed found to be less toxic than pyrethrum.—The insecticidal properties of parts of the mamey tree (*Mammea americana* L.) (*22*) warranted further chemical examination of the material in order to establish the nature of the toxic mass. In this preliminary phase of the work only the powder prepared by grinding the sliced, dry mature seed through the 0.5-mm. sieve of a Wiley mill was studied. This powder was found to be devoid of rotenone-type compounds according to the Durham and red-color tests for such compounds. The marked paralytic action of the powder suggested the presence of pyrethrins or similar compounds.

A prime requisite for work of this nature is a method by means of which the various fractions and derivatives of the original material can be tested to determine the course of separation of the toxic principles. For this purpose liquid preparations were tried without success. It was finally found that a satisfactory method was to synthesize a powder by homogenizing an alcoholic solution of the fraction of which the toxicity was to be determined with inert marc of the original powder. Thus, for example, it was found that mamey seed powder extracted with acetone for 12 hours yielded a marc completely inert toxicologically. When the brown sirupy acetone extractives were dissolved and mixed with the marc at the rate at which they occurred in the original powder and the solvent was evaporated, a powder was obtained having a toxicity comparable with that of the original powder. The various fractions obtained were tested in this manner in the laboratory against susceptible insects as previously reported.

Of several solvents tried, acetone, ethyl ether, benzene, and carbon disulfide readily extracted all of the toxic principle, but petroleum ether, although slow to extract, was the best solvent for the preparation of an extract for chemical examination because it extracted the least nontoxic material. The addition of absorbent carbon to the dark reddish-brown extract in acetone or petroleum ether did not result in purification. Upon evaporation of the solvent from the petroleum ether extractives, a brown sirup remained. Taking this up in either ethyl or methyl alcohol and filtering caused a white waxy precipitate to form in the filtrate. The same precipitate was obtained when the petroleum ether extractives (thoroughly evacuated of solvent) were taken up in glacial acetic acid and a small amount of water was added. This material after recrystallization from either ethyl or methyl alcohol consisted of white, waxy, soft crystals melting at 70° C. It was insecticidally inert, but the brown residue from which the white material was

removed was very toxic. After removal of the fats, waxes, and fatty acids, the residue was highly toxic. The preparation of a semicarbazone from the purified resin was tried, but only very small amounts of brown impure powder were obtained. Hydrolysis of the resins by refluxing 1.5 hours with sodium hydroxide (0.5 N) followed by acidification and extraction with petroleum ether yielded a nontoxic material. Inasmuch as the resin did not contain nitrogen and the toxicity was therefore not due to an amide, this evidence indicated that the toxicity was due to an ester whose component alcohol and acid are nontoxic. A separation of the resin between acetic acid and petroleum ether gave three fractions: Brown insoluble matter, acid soluble fraction, and petroleum ether fraction. When restored to inert marc at the rate of 0.5 and 1 percent, all three showed toxicity comparably with that of the original resin. No semicarbazone could be prepared from these fractions.

Pyrethrin analysis (*5, pp. 66–67*) of the original mamey seed powder and of some powdered pyrethrum flowers showed differences between the two materials. The alkali hydrolyzate of the mamey resin was yellow; that of pyrethrum flowers was colorless. Upon addition of the Denige's reagent the pyrethrin extract became slightly turbid, then changed color from white to pink, red, violet, and blue, all in about 2 minutes. That of mamey, on the other hand, remained yellow but gave a flocculent precipitate of mercurous chloride at the proper time. The pyrethrum flowers contained 0.41 percent total pyrethrins, of which 0.27 was pyrethrin I and 0.14 percent pyrethrin II. The mamey seed powder appeared to contain 0.19 percent total pyrethrins, of which 0.05 percent was I and 0.14 percent II. This, of course, cannot be regarded as a positive test for the presence of pyrethrins.

Several points of comparison and of contrast between mamey seed and pyrethrum flowers have been made. For example, the toxic mass of mamey, hereinafter referred to as mameyin, was concentrated by procedures the same as those used for pyrethrins; it gave positive tests for pyrethrins; its barium salt was shown to be soluble; and it appeared to be an ester. However, by means of a biological comparison of the two it was shown that the toxins were different. This comparison was made as follows: Extracts of mamey seed and pyrethrum flowers were prepared, and aliquots homogenized with mamey seed marc to give powders of several levels of toxicity so that toxicities over the whole range of 0 to 100 percent could be approximated. These powders were then tested in 10 replicates at each level against larvae of *Diaphania hyalinata* (L.) and adults of *Diabrotica bivittata* F. When the data were plotted as concentration against toxicity, both direct and as log v. probit, straight lines were not obtained. However, it could be seen that the concentration-toxicity lines for pyrethrum flowers and for mamey seed were parallel for *D. bivittata* but diverged for *D. hyalinata.* Thus, with the latter test insect, the concentration, in terms of percentage of original resins restored, necessary to give 40-percent toxicity was 13.8 for mamey seed and 2.6 for pyrethrum, signifying that pyrethrum was about 5.3 times as toxic as mamey seed; to obtain 60-percent toxicity required 33-percent restoration for mamey seed as against 4.4 for pyrethrum, thus giving a toxicity ratio of pyrethrum to mamey seed of 7.5. Against *Diabrotica* the pyrethrum was about five times as

toxic as mamey seed at all levels of toxicity. If the toxicity of the two materials were due to the presence of the same toxin, the lines would be approximately parallel regardless of the species of test insect, and the pyrethrum, in accordance with the chemical analysis, would be twice as toxic as the mamey seed. Therefore, it may be concluded from the data obtained thus far that the toxicity of mamey seed is not due to pyrethrins but to another perhaps somewhat similar ester.

PLANT TOXICOLOGICAL STUDIES

By Harold K. Plank

Several plants tested possessed mild insecticidal properties.—Exploratory tests for insecticidal possibilities were carried out with pulverized seeds and pods of six introductions of two varieties, A and C, of *Pachyrhizus erosus* (L.) Urban. Also included were like parts of variety A of *P. palmatilobus* (Moc. & Sessé) Benth. & Hook. On account of the high oil content of the seeds, an equal part of the corresponding pods, which with one exception were inert to all the test insects tried, had to be added to facilitate grinding. Such 50–50 dusts of *P. erosus* var. A, C 43–10, and M. No. 7208 from Mexico were highly toxic, 95.2 percent, to the melon worm (*Diaphania hyalinata* L.), but less than 80 percent toxic to the fall armyworm (*Laphygma frugiperda* (A. & S.)) and larvae of the diamondback moth (*Plutella maculipennis* (Curt.)). Variety C and the remainder of the introductions of variety A showed appreciable toxicity, up to 88 percent, to the first two of these insects and in some cases to one or two other insects. *P. palmatilobus* var. A was 76 percent toxic to the melon worm and 80 percent toxic to the fall armyworm. It is important to note that none of the introductions of either species were toxic to a local bean leaf beetle, *Cerotoma ruficornis* (Oliv.).

The mature fruit, i.e., seeds in pods, of *Aeschynomene sensitiva* Sw. showed a toxicity of 82 percent to the melon worm but was inert to the fall armyworm and *Cerotoma* adults. The seeds of this plant were previously found by Jones (*14, p. 14*) to contain rotenone-type compounds equivalent to 0.08 percent of rotenone and the pods the equivalent of 0.18 percent. In comparison it is interesting to note that laboratory tests of a sugarcane-bagasse dust of the same fineness impregnated with derris root extract to contain 0.25 percent of rotenone showed a toxicity of 26 percent to the melon worm, 13 percent to the fall armyworm, and none to *Cerotoma* adults. The seeds of *Calopogonium coeruleum* (Benth.) Hemsl. gave a control of 63 percent when tested against the fall armyworm and 26 percent against the melon worm, but were inert to the adults of *Cerotoma*; the pods showed a maximum toxicity of 35 percent. Immature and nearly ripe fruits of *Gliricidia sepium* (Jacq.) Steud. were almost inert, possessing less than 18-percent toxicity, and a 20–80 mixture of seeds and hulls and the hulls, leaves, bark, and wood of *Calophyllum antillanum* Britton showed 40-percent or less toxicity. The bark of *Canella winteriana* (L.) Gaertn., a plant said to be used locally for stunning fish, was 64 percent toxic to adults of *Cerotoma*, but the wood was ineffective against this insect and all others tried. The immature and ripe fruits of *Cassia alata* L. were 46 percent and 58 percent toxic, respectively, to the melon worm. The leaflets,

petioles, bark, and wood of this plant and of *C. spectabilis* DC. and the immature and ripe fruits and leaves of *Solanum ciliatum* Lam. (cockroach berry) were practically inert.

When used against a cotton stainer, *Dysdercus andreae* (L.), laboratory tests revealed a maximum toxicity of 19 percent for the powdered mature seeds of mamey and of 33 percent for a 50–50 dust mixture of the seeds and pods of introduction C 43–22 of var. A of the yam bean (*Pachyrhizus erosus*). Other parts of mamey, the seeds of *Aeschynomene sensitiva*, parts of *Calophyllum antillanum*, the immature and nearly ripe fruits of *Gliricidia sepium*, and the additional introductions of the yam bean mentioned previously showed considerably less or no toxicity against this insect or the rice weevil (*Sitophilus oryza* (L.)). In these tests the usual procedure was modified to the extent that the cotton stainers were rolled in the dust and then confined with sliced pods of maga (*Montezuma speciosissima* Sessé & Moc.), whereas the rice weevils were confined in half-pint screw-top jars containing 10 gm. of corn with which 0.1 gram of the dust being tested had been thoroughly mixed.

Mamey seed has possible insecticidal value for local use.—Powdered mamey seed, 8 pounds in 100 gallons of water, and 40-percent nicotine sulfate, 1 part to 800 parts of water, were applied in February with a hand sprayer to potted 6-week-old cabbage plants of two varieties for the control of aphids. Powdered soap, 4 pounds per 100 gallons of water, was used as a spreader. Four days after spraying, the aphid population was reduced 72 percent by mamey, 96 percent by nicotine sulfate, and 50 percent by the soap spreader alone.

The application of the above materials, along with a freshly mixed dust containing 90 percent of powdered mamey seed and 10 percent of hydrated lime, was applied to 2-month-old cabbage plants in the field for the control of aphids and the diamondback moth. Although too few aphids were present for the results to be significant, it was noted that under these conditions the mamey-lime dust appeared to be better than the mamey-soap solution and both were inferior to nicotine sulfate. This single application resulted in a statistically significant reduction of the diamondback moth population of 48 percent by mamey-lime, 69 percent by mamey-soap solution, and 71 percent by nicotine sulfate. These last two materials gave results similar to those previously obtained in a preliminary test on cauliflower in which a proprietary spreader was used instead of soap.

Considerable injury to the leaves by the above liquid sprays, particularly by the mamey-soap combination, began to be noticeable on most of the cabbage plants the second day after application. The injury occurred as irregular, rough, wartlike patches up to several square centimeters in area, chiefly on the underside of the leaves. On some plants, the epidermis was killed on the midrib and other parts exposed to strong sunlight. Apparently the reaction of the mamey with the powdered soap used was responsible, for no injury was noticeable on any of the plants dusted with the mamey-lime mixture.

Four applications at 10-day intervals of mamey seed dusts diluted with lime and with talc to contain 90 percent of mamey and a derris dust with the same diluent to contain 0.5 percent of rotenone were tested against the diamondback moth on cabbage and broccoli. On cabbage the in-

festation was variable and control by any treatment was not very effective. On broccoli the larvae were better exposed, and mamey-lime showed 42-percent control, mamey-talc 55-percent, and derris-talc 75-percent. That mamey was less effective mixed with lime was probably because the lime, in the presence of dew on the plants, hydrolyzed some of the toxic resins. Practically no decrease in toxicity against the larvae of this insect was noted with the same material, or with mamey-talc, stored dry and tested dry in petri dishes at frequent intervals up to 2 months after mixing.

A kerosene extract of mamey seed prepared by soaking ½ pound of the powder for 24 hours with intermittent agitation in 1 quart of kerosene at room temperature and filtering was tried against several insects, using a small hand sprayer. Considerable kill was obtained of cockroaches, mostly *Periplaneta americana*(L.), flies (undetermined), ants, principally *Prenolepis longicornis* Latr., mosquitoes (undetermined), and adult drywood termites, *Cryptotermes brevis* Walker. This extract contained only a small part of the active principle in the mamey seed, for, after evaporation of the kerosene solvent, the marc was found to be nearly as toxic as the original powder.

All parts of the desert date toxic to snails.—Plants of the desert date (*Balanites aegyptiaca* (L.) Delile), a fish-poison plant, grown from seeds received in 1936 from Palestine, South Africa, and Kenya Colony, were planted on the banks of the Vives Irrigation Reservoir near Guayama in 1937. This original introduction of *B. aegyptiaca* into Puerto Rico was made by this station at the suggestion of W. A. Hoffman [5] of the School of Tropical Medicine of the University of Puerto Rico. At that time Dr. Hoffman, a parasitologist studying schistosomiasis, and C. L. Horn, horticulturist of this station, selected the site at Guayama, and the planting was made in cooperation with the owners, Luce and Co., Aguirre, P. R. Of 76 trees originally planted, 43 now remain, which range from 5 to 15 feet in height. These trees were reported to have first flowered in 1941 and to have had the heaviest fruit set to date in January 1945.

The fruit and bark of species of *Balanites* have been known for a long time in Nigeria and in India to have fish-killing and insecticidal properties, [6] and in Egypt *B. aegyptiaca* has been used to control the molluscan hosts of the liver fluke, *Schistosoma mansoni* Sambon, that infests man and certain other animals (*9, pp. 18–19*). The fruit is reported to be highly toxic also to the cercariae and miracidia, the free-swimming stages of the fluke, even at high dilutions (*3, p. 208*). Schistosomiasis, or bilharzia, as the disease is commonly known, is prevalent in Puerto Rico. Although no exact figures are available, it is known that the rejection of army inductees has been high because of infection with this disease.

To secure some idea of the toxicity under local conditions to the snail *Australorbis glaboratus* (Say), the only known alternate host of *Schistosoma mansoni* in Puerto Rico, laboratory tests were made of material from the trees of *Balanites aegyptiaca* at Guayama. Various parts were

[5] Deceased.

[6] Roark, R. C. EXCERPTS FROM CONSULAR CORRESPONDENCE RELATING TO INSECTICIDAL AND FISH-POISON PLANTS. U. S. Bur. Chem. and Soils. 39 pp. 1931. [Processed.] (See p. 4.)

finely cut or mashed and added to unpurified pond water in 2-quart jars, after which five snails were placed in each jar. Each treatment and the control was replicated five times. At a concentration of approximately 1 part to 860 parts of water by weight, the shell and underlying pulp of the yellow-ripe and green-ripe fruits killed 100 percent of the full-grown snails in 48 hours. At a concentration of about 1 to 2,600, the partly dried bark killed 96 percent and the fresh root 100 percent of the same stage, and the partly dried leaves killed 72 percent of somewhat smaller snails in the same length of time. In about 4 hours after exposure, blood was seen streaming from some snails in the jars containing the two kinds of fruit pulp. In 48 hours considerable sourness had developed in these jars, and bleeding was noted in all except the controls. In a week all treated snails were dead except three in the jars containing leaves, while only one snail was dead in the controls.

The Vives reservoir at Guayama contained a high population of the snail host of *Schistosoma mansoni*, and the incidence of schistosomiasis in that region was said to be among the highest of the island. The owners of the reservoir stated that fruits falling into the water since the trees began to bear several years ago have caused some reduction in the snail population. Counts made in 2 areas of 1 square yard each in each of 2 locations when the reservoir was nearly empty showed 15 and 18 percent of the snails dead at 15 feet from the shore line, and 72 and 75 percent dead at 4 feet from the shore line, or about these distances from overhanging limbs of bearing *Balanites* trees. The areas examined were on the lee side of the reservoir, where the wind tended to accumulate floating dead snails in numbers that increased as the shore line was approached; there were no trees and practically no snails on the windward side. All 4 counts, totaling 324 snails, showed an average mortality of 50 percent. A count of 151 snails in an area of about 2 square yards in a small drained pond on the station grounds at Mayaguez, where no *Balanites* trees were growing, showed a natural mortality of 31 percent. Only 1 small dead fish was seen in the above reservoir as compared with large numbers of living fish, some swimming near fruits that had fallen into the water.

DRUG-CROP INVESTIGATIONS

CINCHONA PROPAGATION

By Roy E. Harper and Harold F. Winters

Cinchona field plantings total more than 6,000 trees.—Field plantings of *Cinchona* during 1944 were made largely in the Toro Negro Forest area on land that had been cleared of most of the forest growth and prepared for planting 4 to 10 months previously. Other small adaptation trial plantings were made in the Maricao and Luquillo Forests, and a few trees were given to two local farmers, one near Lares and the other near Jayuya. Table 3 gives a summary of the field plantings made in 1943 and 1944 and the number of trees surviving as of June 1, 1945.

For the field plantings of 1944, the land was cleared, terraced, and ditched during the previous winter and spring. This early preparation gave an opportunity for weeds to cover the soil before the rainy season, thereby minimizing soil erosion.

TABLE 3.—*Cinchona field plantings made in Puerto Rico in 1943 and 1944 and number surviving June 1, 1945*

Location, elevation, and rainfall	Species	Date planted	Number planted	Number surviving	Percent surviving	General condition
Toro Negro Forest—elevation, 3,200 ft.; average annual rainfall, 120 in.	*Cinchona ledgeriana* Moens, progeny of 6 trees in Maricao planting	Aug.-Nov. 1944	1,523	1,372	90.1	Good
	C. ledgeriana, Guatemala, Coban	Nov.-Dec. 1944	1,384	1,060	76.6	Fair to good
	C. ledgeriana, P. I. No. 143981	Sept.-Oct. 1944	2,202	735	33.0	Poor to fair
	Cinchona sp. (mostly hybrids)	Oct.-Nov. 1944	898	816	90.1	Good
	C. pubescens Vahl, Guatemala	Aug. 1943	100	22	22.0	Fair
	C. ledgeriana, Maricao progeny	Aug.-Oct. 1943	1,500	295	19.7	Fair to good
Maricao Forest	*C. calisaya josephiana* Weddell. Castañer	Nov. 1944	93	85	91.4	Good
	C. ledgeriana, Guatemala	Nov. 1944	182	106	58.2	Fair
Luquillo Forest—elevation, 2,000 ft.; average annual rainfall, 170 in.	*C. ledgeriana* and *C. pubescens*	Jan. 8, 1945	105	85	80.9	Fair
Hacienda Santa Barbara, Jayuya, P. R.—elevation, 2,500 ft.; average annual rainfall, 80 in.	*C. ledgeriana*	Oct. 1944	50	0	0	----------
Bauzá Finca, Lares—elevation, 1,500 ft.; average annual rainfall, 97 in.	*C. calisaya josephiana*	June 1943-Sept. 1944	60	48	80.0	Good
	C. hybrids, Castañer	June 1943-Sept. 1944	30	8	26.7	Fair

Results of the initial *Cinchona* field plantings of 1943 at Toro Negro indicated that trees planted after the period of heaviest rainfall survived and grew better than those planted earlier. In order to get more information on the optimum season for field planting, the 1944 plantings were begun August 15, after the beginning of the heavy rains, and were continued intermittently through the following 4½ months. The lower mean temperatures during November and December tend to produce slower growth, making the trees hardier for transplanting, and may tend also to offset the lighter rainfall as it affects humidity and soil moisture. It is apparently necessary to be more critical of the weather factors when field planting is done during the summer months than when it is done later in the season. For example, planting should not be continued through short periods of dry weather and it is disadvantageous from an operations standpoint to plant during periods of heavy rains. In about half the area planted during the past season the land was completely cleared of forest trees before preparation. At the time of planting a satisfactory temporary shade was made by staking two or three leaves of the "sierra" palm (*Euterpe globosa* Gaertn.) around the base of the tree and tying them together over the top. The shade was removed after 1 to 2 months when the trees had started growth. Survival has been satisfactory under this method, and the trees appear to be growing better than those planted under natural shade.

In the individual planting pits, the soil was opened to more than 1 foot in depth, and organic materials in the form of forest leafmold and

duff were added. The sites were then mounded and allowed to settle a few months before planting, which permitted planting the trees at the proper soil level without danger of excessive settling which would leave them in a hole to catch surface water.

Adequate wind protection is also important. The principal damage is done by persistent winds which blow the stems of newly planted trees around until the roots are loosened and broken and the base forms a hole in the soil which catches water and increases the incidence of disease. Results at Toro Negro indicate that exposed slopes may be utilized to some extent for planting *Cinchona* if areas of ½ to 1 acre each are blocked off by strips of the forest trees for wind protection. In some areas windbreaks of natural tree growth will be most effective if left along the principal ridges.

Leaf composts were superior to mor as source of organic material in the *Cinchona* nursery. Mor, or forest duff, has been the most convenient and plentiful source of soil organic matter for use in the *Cinchona* nurseries at both Maricao and Toro Negro. Other materials have been tried on a small scale but usually without results that were superior to the commonly used mor. As reported previously (*10, p. 5*), forest debris containing appreciable amounts of composted woody materials was definitely inferior for use in the soil mixture.

When new nursery beds were constructed at Toro Negro in June and July 1943, one plot was prepared with a 3- to 4-inch layer of leafmold in the bottom, one with forest debris mixed with soil, and one contained soil alone. In the first-mentioned plot seedling growth was somewhat better than that in the other plots, and the roots of the young *Cinchona* trees dug for transplanting were well-developed and remarkably free of disease.

At Maricao an accumulation of old palm leaves composted for 1 year was used as a nursery soil mixture for comparison with mor. Both organic materials were mixed with coarse loamy sand in a ratio of 1: 1. Seedlings were transplanted to the plots in February 1944. There was no perceptible difference in survival or growth during the dry season between the plants in the two soil mixtures. After the beginning of the rainy season in July, however, the common root symptoms of poor drainage appeared in the plots containing mor in the soil mixture. By the end of September more than 40 percent of the seedlings in these plots had become diseased, whereas in the palm leaf compost mixture less than 5 percent of the plants appeared to be diseased. Growth among healthy plants was also superior in the latter soil.

Highly acid nursery soil was detrimental to survival and growth of *Cinchona* seedlings. An experiment was conducted at Maricao to determine the effect of low pH of nursery-bed soils on the survival and growth of *C. pubescens*. A sand-duff or sand-duff-peat mixture of soil was used, with wooden partitions to separate the plots, which contained 50 plants each spaced 6 inches apart. The pH was lowered by use of pulverized sulfur in amounts shown in table 4. Gypsum was applied in another treatment since it contains sulfur but does not alter the pH. Soil samples for determination of pH were made with a glass-electrode pH meter.

Height measurements were made of all plants, and the average height for treatments and replications are shown in table 4. Survival was

poorest and size of the plant smallest in treatment No. 4, where 2 pounds of sulfur were added to each plot of the sand-duff mixture. Two replications out of the three lacked data for this treatment, and, therefore, it was not used in an analysis of variance. However, the data on height of plants in the remaining treatments were analyzed, and significant differences found between treatments. This analysis indicates that treatment No. 3, receiving 1 pound of sulfur per plot, with a mean height of 8.45 cm., was inferior to all others except that receiving 2 pounds of sulfur.

TABLE 4.—*The effect of pH of nursery soil on height, leaf area, and survival of Cinchona seedlings (50 plants per plot, 3 replications)*

Treatment	Range in pH	Mean height	Mean leaf size index	Mean surviving plants
		Cm.	*Cm.*[3]	*No.*
1. Sand-duff, 1–1 (control)	4.70–4.83	12.93	4.11	35.00
2. Sand-duff, 1–1, ½ lb. sulfur	2.76–3.33	12.38	3.68	32.33
3. Sand-duff, 1–1, 1 lb. sulfur	2.61–2.93	8.45	3.07	11.33
4. Sand-duff, 1–1, 2 lb. sulfur	2.40–2.56			
5. Sand-duff, 1–1, 2½ lb. gypsum	4.28–4.92	13.25	4.24	31.33
6. Sand-duff-peat, 2–1–1	4.62–4.83	12.38	4.03	29.67
7. Sand-duff-peat, 2–1–1, ½ lb. sulfur	2.92–3.56	12.56	3.85	32.33
8. Sand-duff-peat, 2–1–1, 2½ lb. gypsum	4.46–4.70	12.71	3.86	31.33

Measurements of length and width of the largest leaf on each plant also were made. The values obtained by multiplying the length by the width were then reduced by taking the cube root of each item, and the average leaf size values were then analyzed for variance. A third analysis of variance was made using the numbers of plants surviving. Both of these latter analyses likewise showed that plants receiving 1 and 2 pounds of sulfur were inferior to plants in other treatments.

Imported nursery seedlings made good growth at Toro Negro.—The seedlings of *Cinchona ledgeriana* stock collected by Col. A. F. Fischer in the Philippines and grown at Glenn Dale, Md., have made fair to good growth during the past season in the Toro Negro Nursery. The trees now average 1½ feet or more in height. Of 8,250 trees planted in January and February 1944, there remain approximately 4,726 trees, a survival of 57 percent. An approximate 20-percent loss of seedlings was caused by an infestation of thrips during September and October, but these insects were brought under control by the application every 2 weeks of a dust containing 1 percent rotenone. Seedlings which were slightly damaged have resumed normal growth.

A collection of 30 special strains of *Cinchona* made by Colonel Fischer has been grown from seed in the Maricao seedbeds and placed in nursery beds at Toro Negro. Plantings made during December and January are now growing satisfactorily, but those made during February have suffered because of the dry conditions and windy weather prevailing at the time of planting.

GENETICAL STUDIES

By Roy E. Harper

A study of seedling variation in certain strains of Cinchona ledgeriana.—Since *Cinchona* is naturally a cross pollinator (*10*), it might be

expected to be preponderantly genetically heterozygous. It was found that practically all seedling progenies of *C. ledgeriana* vary in a large number of characteristics such as vigor of growth; branching habit; twig size; leaf size, color, and shape; and pubescence. Some differences were found between progenies or strains in character distributions, but the main varietal characteristics appear to have fairly definite limits of variation.

When *Cinchona ledgeriana* is crossed with *C. pubescens* the hybrid closely resembles the *C. pubescens* parent with a masking effect on the expression of the principal *C. ledgeriana* characters. In the second hybrid generation, or F_2, some unmasking is found with the expression of various combinations of *C. ledgeriana* characters appearing.

The Philippine introduction made by Col. A. F. Fischer, P. I. No. 143981, is presumably a composite of several *Cinchona ledgeriana* progenies as evidenced by the wide variation in characters. In addition, some 10 to 15 percent of the seedlings show evidence of hybridization with *C. pubescens*. These have generally grown more rapidly than *C. ledgeriana* types and have larger stems, with larger, broader leaves. About one-fourth of them appear to be first-generation hybrids, with extremely rapid growth and *C. pubescens*-like leaves. The remainder are either second-generation hybrids or backcrosses to *C. ledgeriana*. A preliminary study of this strain has indicated that about the most reliable character on which to base hybridity is width of leaves. In leaf measurements of *C. ledgeriana* seedlings it was found that the ratio of width to length ranges from 0.25 to 0.40. The same ratio among hybrids ranges from about 0.35 to 0.60. All types having leaves greater than 10 cm. in width can be considered to be hybrid in composition.

CINCHONA INSECT INVESTIGATIONS

By Harold K. Plank and Harold F. Winters

Red spider and other insects fed on Cinchona.—A species of *Tetranychus* [7] was found in June and November attacking the underside of the leaves of small *Cinchona* seedlings growing in flats in the greenhouse at Mayaguez. Severe feeding caused the leaves to wilt and die. Complete control was obtained by one application of dusting sulfur.

A small brown thrips, *Anaphothrips* (*Chaetanaphothrips*) *orchidii* (Mlt.), [8] found last year at Maricao, appeared in October on the leaves of young seedlings in the greenhouse. Repeated applications of derris dust containing about 1 percent of rotenone gave good control, as did also nicotine sulfate, 1–800 plus 1 percent of an emulsifiable white oil, but the former method was the more practicable. The greenhouse thrips (*Heliothrips haemorrhoidalis* (Bouché)) [8] was discovered in September feeding on the leaves of seedlings of *Cinchona calisaya* in nursery beds at Maricao. Some of the plants were seriously attacked from the underside causing curling and dropping of leaves. Older trees in the same area were also infested and in some cases partly defoliated.

The two foregoing species of thrips and another species, *Scirtothrips longipennis* Bagn., reported last year from Maricao, were found in September and October on seedlings at Toro Negro. In addition to the

[7] Determined by E. A. McGregor, Bureau of Entomology and Plant Quarantine.
[8] Determined by J. C. Crawford, Bureau of Entomology and Plant Quarantine.

same control treatments that were used in the greenhouse at Mayaguez, tartar emetic at the rate of 4 pounds to 100 gallons of water plus 16 pounds of sugar was tried. Of these, frequent applications of the derris dust gave the best results.

The lily aphid (*Myzus circumflexus* (Buckt.)) [9] and the black citrus aphid (*Toxoptera aurantii* (Fonsc.)) [9] were discovered in moderate numbers in late October on the underside of the small center leaves of year-old seedlings in nursery beds at Maricao. Between 5 and 10 percent of the plants were attacked, causing the leaves to curl and wrinkle abnormally. Sporadic aphid infestations have also been seen in the nurseries at Toro Negro. However, neither species was observed to gain headway or to cause any appreciable damage.

Larvae of the sphingid, *Xylophanes pluto* (F.) [10] were found at various times from May to December feeding extensively on the margins of the leaves of seedling trees in the Maricao nursery. These larvae were never numerous, and damage was prevented by hand picking.

Certain strains of Cinchona appear to be partly resistant to thrips.—Owing presumably to a period of unusually dry weather, thrips population caused serious damage in the *Cinchona* nurseries at both Maricao and Toro Negro. Certain progenies and strains of *Cinchona*, although about equally exposed to infestations of thrips, seemed to escape damage. Two progenies from Maricao parent trees are among those that seem to "outgrow" the thrips after they reach the nursery stage.

Some types of seedlings evidenced some resistance to thrips infestation. For example, one large nursery plot at Toro Negro was divided into two parts, and one part was planted to greenhouse-grown seedlings that were tall and spindly in growth. The other part of the plot was planted with a group of seedlings from the Maricao seedbeds that were comparatively small for their age and somewhat stocky in development. Seven months after transplanting, the tall seedlings were heavily infested, with 15 to 20 percent mortality due to thrips injury. The small seedlings were lightly infested at several points but showed little checking of growth. This pronounced difference in susceptibility, somewhat greater than that observed between different progenies grown under the same conditions, might be attributed to other factors. The slower growth of the disproportionately tall seedlings after transplanting may have caused them to be more susceptible to injury or the greater humidity around the foliage of the more compact plants was unfavorable for thrips development.

FOOD-CROP INVESTIGATIONS

SEED PRODUCTION

By David G. White

Seed of sweet corn and soybeans grown to increase local food production.—In cooperation with the War Emergency Program of the Insular Government, sweet corn and soybean seed were grown to increase local food production. During the year 2,054 pounds of USDA–34 sweet corn, 3,581 pounds of the Seminole variety of soybeans, and 8,562 pounds of yams (*Dioscorea* sp.) were furnished for distribution.

[9] Determined by P. W. Mason, Bureau of Entomology and Plant Quarantine.
[10] Determined by Carl Heinrich, Bureau of Entomology and Plant Quarantine.

VEGETABLE INVESTIGATIONS

By Norman F. Childers, Harold F. Winters, Pedro Seguinot Robles, and Harold K. Plank

Vegetable-variety trials initiated at three altitudes.—Comparatively little information is available on the best vegetable varieties to plant in Puerto Rico, especially at the altitudes where temperatures are low. In January 1945 a vegetable program was initiated to determine the adaptability of certain vegetables at Mayaguez (50 feet), Maricao (2,000 feet), and Toro Negro (3,300 feet). The program extends over a period of 2 years and involves approximately 45 vegetables, half of which will be tested the first year. Plantings are being repeated at 2-month intervals until each vegetable has been tested over a 12-month period. Vegetables selected for first trial at the higher altitudes consisted largely of cool-season crops, whereas those at Mayaguez were both cool- and warm-season crops.

Most vegetables did well at Mayaguez during the dry season.—The vegetables which gave best development and yields when planted about the first of February were eggplant, cucumber, onion, leaf lettuce, Chinese cabbage, squash, radish, tomato, and okra.

The New Long White Bunching variety of onion appears to be well adapted to Mayaguez conditions and can be definitely recommended as a bunching onion which is relatively resistant to onion thrips (*Thrips tabaci* Lind.). The Crystal Wax Bermuda and the Yellow Bermuda bulb onions, although not particularly resistant to thrips, were satisfactory and the best producers of the nine varieties tested. The quality of the radishes and lettuce was excellent. The Black Seeded Simpson leaf lettuce produced about three times as much foliage in a given time as Mignonette, the leaves of the latter being of fine quality, but had a dull greenish-red color, which is considered unattractive on the Puerto Rican market. Mignonette is actually a head lettuce, but no heads were formed under Mayaguez conditions. The Imperial 44 and Great Lakes head lettuce developed heads, whereas Imperial 847 and Improved Florida Iceberg 847 developed flower stalks. The heads of both cabbage and lettuce were salable but small, soft, and light in weight, 1 to 2.2 pounds. The better varieties of cabbage were All Head Early, Henderson's Succession, Stein's Early Flat Dutch, and All Seasons. The heads of Golden Acre showed considerable splitting. The most tender and attractive Chinese cabbage was the Pe Tsai variety. Chi Hi Li grew well, however, as did Wong Bok.

Failure of the beets and carrots was due to the crust formed by the heavy clay soil, which interfered with germination and early growth, and nutgrass was a serious weed problem. Satisfactory beets and carrots, however, were germinated and grown nearby on a lighter sandy soil.

The yield of 9.0 pounds of fruit per plant for the Pritchard tomato is worthy of note under Mayaguez conditions. This is a better yield for tomatoes than obtained at Maricao or Toro Negro. Blight was not a serious problem where liquid bordeaux was applied weekly. However, mosaic was present in all plants at the conclusion of the trial and unquestionably lowered the total yields.

Eleven Hawaiian hybrid varieties of tomatoes, furnished by the University of Hawaii Agricultural Experiment Station, were tested in a sepa-

rate trial, and all grew satisfactorily. The indeterminate varieties grew to a height of more than 6 feet when trained on bamboo poles. The four highest-yielding varieties were T. H. 921, 7.2 pounds of marketable fruit per plant; C2, with 6.0; Bounty (Hawaiian), 5.1; and T. H. 927, 4.8 pounds.

Irrigation was provided for all crops during dry periods by a portable oscillating sprinkler system.

The most persistent insects were the diamondback moth on cole crops, aphids on lettuce and cabbage, and cutworms and mole crickets affecting nearly all seedlings. Damage from the latter two insects was reduced to less than 1 percent by placing a 1–30 mixture of paris green and corn flour in circles about the young plants or in strips adjacent to the rows. Leafhoppers (*Empoasca fabalis* De Long) are such a problem with beans that only a few varieties can be suggested. King of the Garden pole lima, Puerto Rico Bush White No. 1160, and Giant Stringless Greenpod bush bean were comparatively resistant to this pest.

Cole crops did well at higher altitudes.—At Maricao the most satisfactory vegetables grown from plantings made in February were broccoli, cabbage, collard, kale, kohlrabi, and parsley. Heads of cabbage averaged between 3 and 4 pounds. Vegetables which gave fair results were endive, peas, tomato, rutabaga, and a variety of cauliflower, known as Early Market, from the Hawaiian Sugar Planters' Association. It should be pointed out that any or all of these vegetables may have performed somewhat better with special or modified practices. Wind definitely damaged the transplanted vegetables, and it was necessary to stake tall plants, such as broccoli, to prevent them from twisting in two. Wind may have been an important factor also with head lettuce, spinach, and radish, which grew poorly.

It is interesting to note that a local vegetable grower near Maricao has grown cauliflower (Burpeana) and head lettuce (Imperial 44) with somewhat more success on Catalina clay supplied with liberal quantities of goat manure and commercial fertilizer. The heads of lettuce weighed between 2 and 3 pounds. The station garden at Maricao is located on Nipe clay, which is high in iron (about 50 percent Fe_2O_3), chromium, and nickel and low in phosphorus and potash. It is a relatively unproductive soil, but when cultivated it does have good aeration.

At Toro Negro, cole crops such as cabbage, broccoli, collard, kale, and kohlrabi grew best and were of excellent quality at this higher elevation of 3,300 feet from February to May. The heads of cabbage were far better than those grown at Mayaguez and Maricao. Their average weight was between 3.5 to 5.5 pounds, with some heads attaining 9 pounds. An excellent crop of radishes was harvested within 30 days of planting. The texture was fine and crisp and the flavor mild.

The vegetative growth of peas was definitely correlated with the cooler temperatures at the higher altitudes. For example, the Telephone variety grew to about 8 feet at Toro Negro, 4 feet at Maricao, and 2 feet at Mayaguez. Yield of peas likewise was correlated with altitude for both the Telephone and Melting Sugar varieties, the latter being the more productive.

Endive, turnip, parsnip, parsley, rutabaga, and carrot gave good results at Toro Negro. However, growth of tomatoes and head lettuce

was definitely unsatisfactory. The Pan America tomato survived better than Marglobe but grew only to about 2 feet and the leaves were small and bluish purple, probably as a result of the cold night temperatures (about 55° F.). The small lettuce heads may have been due to insufficient nitrogen or to persistent winds.

The diamondback moth caused trouble with the cruciferous plants, and late blight was a problem with tomatoes, but both were kept in check by frequent sprayings.

TROPICAL FRUITS

By Milton Cobin

Direct seeding of larger mangosteen seeds gave highest germination and survival.—An experiment was started in 1943 to determine whether the size of the seed of *Garcinia mangostana* L., as indicated by its weight, affected vigor and whether the size of the seedling at the time of transplanting, as indicated by the number of leaves, affected its subsequent development. The 6-month data were reported last year (*8, pp. 23–24*).

Between the 6- and 10-month periods the least mortality was observed in those seedlings whose seeds were sown directly in nursery beds. In the case of transplants, those in the two-leaf stage showed the only appreciable increase in mortality and this was among seedlings of the lower weight class. Among the four-leaf transplants only those from the heavy seed-weight class showed a higher percentage of survivors at 10 months than at 6 months. In the six-leaf transplants, each weight class had a higher percentage of surviving transplants recorded for the 10-month period than the 6-month period, yet the heaviest weight class had the lowest total percentage of survival of all seeds sown. This was attributed to the greatest percentage of transplants made in this weight class than in the others and indicated the desirability of transplanting at an earlier stage to prevent damage. At the end of 10 months the greatest survival of all seedlings transplanted was from the plants obtained from seeds of the heaviest weight class. Distinct differences in rates of growth were recorded for each weight class as measured by leaf count, height of plants, and length of the largest mature leaf. Leaf-width measurements were less indicative of plant vigor, as many of the plants were in the two-leaf stage and leaves of the first pair are usually much wider than those of the next few pairs. The data indicated that where moisture and drainage conditions can be controlled, direct planting to nursery beds of seeds weighing 1 gram or more gave better results than planting the seeds in pots and transplanting the young trees to nursery beds during early seedling stages.

During June 1944, 500 normal, well-formed seeds of *Garcinia mangostana* were collected from the fruits of trees on the station grounds. These were weighed and planted in individual 1-gallon containers in a shaded greenhouse. Three hundred seeds were planted in a mixture of one-third peat and manure and two-thirds Toa sandy loam by volume; the other 200 were planted in a mixture of 90 percent peat and 10 percent manure. The weight distribution and the number of seeds that germinated were as follows: 24.6 percent of the seeds weighed 0.6 gram or less, and of these only 26.8 percent germinated; the other 75.4 percent of the seed weighed 0.7 gram or more and 87.8 percent of these germi-

nated. It was apparent from these results that percentage of germination was directly correlated with the weight of seed.

PLANT INTRODUCTION AND PROPAGATION

PLANT INTRODUCTIONS

By David G. White, Milton Cobin, and Edward P. Hume

A rubber breeding garden established.—In cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering plans were made for the establishment of a breeding garden of *Hevea brasiliensis* (H. B. K.) Muell. Arg. at the station. More than 200 2-year budded stumps representing 24 far eastern clones were received from Marfranc (Jeremie), Haiti. The eventual layout of the garden provides the greatest number of possible combinations with 12 of the highest-yielding and most disease-resistant Ford-Brazilian clones yet to be added.

Shipments of seeds and plants representing 105 species were received from Bermuda, Canal Zone, Canary Islands, Chile, Cuba, Ecuador, England, Haiti, Hawaii, Italy, Liberia, New Zealand, Tahiti, and the United States.

Desmodium nicaraguense Oerst. a leguminous woody shrub or small tree reported to be high in protein, was received from El Salvador, where it is found, normally growing at about 3,000 feet. Planted at Toro Negro on Ciales clay loam, it has shown good initial growth and at Mayaguez has flowered and seeded. The possible importance of this plant to Puerto Rico would be in its use as a source of high-protein pasture. An analysis showed that the leaves had 26.3 percent protein on a dry basis.

Seeds of *Psychotria bacteriophila* Valet. were obtained from the Bureau of Plant Industry, Soils, and Agricultural Engineering. This interesting rubiaceous shrub produces bacterial nodules on the foliage and, when grown in pots, the leaves were found to contain 2.57 percent nitrogen. Plants of this species have been planted at Toro Negro to be tested as a possible source of high-nitrogen mulch.

Six varieties of tangelo, three of orange, and two of lime were received from the Plant Introduction Garden, Glenn Dale, Md. Seeds of 13 species of ornamentals were received from the Canary Islands.

Seeds of highland Chilte, *Cnidoscolus elastica* Lundell; Colima Chilte, *Cnidoscolus* sp. (undescribed); and lowland Chilte, *C. tepiquensis* (Cost. & Gall.) McVaugh, were received through the New York Botanical Garden in cooperation with the Chicle Development Co. from Mexico. About 300 of these trees were planted in the Guanica Forest area. Other plants were set at Mayaguez, Las Mesas, Maricao, and Toro Negro in order to observe the effects of different altitudes and climatic conditions. Chilte has economic possibilities as a source of chicle and is reported to grow well in dry areas such as the south coast of Puerto Rico, where new crops are badly needed.

PLANT DISTRIBUTIONS

By David G. White, Victor Valle, Edward P. Hume, and Milton Cobin

Shipments of plants made to 22 foreign countries.—Plant distributions for the year totaled 55,691 plants, 25,617 seeds, 392 square feet

of grass sod, and 54 pounds of grass runners. These included seeds and plants shipped to Brazil, Canary Islands, Ceylon, Cuba, Dominican Republic, Ecuador, Guatemala, Haiti, Hawaii, Honduras, Jamaica, Liberia, New Guinea, Nicaragua, Palestine, Panama, Peru, Spain, St. Croix, St. Thomas, Tanganyika Territory, and Trinidad.

The military bases on the island received a considerable quantity of plant material from the station, totaling 6,572 plants, 1½ pounds of seed, 190 square feet of grass sod, and 11, 630 feet of bamboo culms.

PROPAGATION STUDIES

By David G. White, Norman F. Childers, and Aida G. Villafañe

Manila grass responded to nitrogen fertilizers.—An area of Manila grass (*Zoysia matrella* (L.) Merr.) was established near the new wing of the station building on what is probably subsoil. This grass was growing poorly and had a yellowish-green color in comparison with other areas. Calcium superphosphate at the rate of 400 pounds per acre and hydrated lime at the rate of 2 tons per acre were applied separately as surface dressings but did not cause any noticeable response 5 weeks after application. Potassium chloride at the rate of 200 pounds per acre also gave no response. However, nitrogen in the form of potassium nitrate applied at a rate of 250 pounds per acre gave a marked improvement in green color and growth within 2 weeks after treatment. Nitrogen in the form of a watery extract of cow manure showed only a moderate response, but this was probably due to insufficient quantity of the extract.

Short days found conducive to seeding of Manila grass.—The production of seed by *Zoysia matrella* appeared to be associated with the length of day when the photoperiod was artificially regulated. Grass sod, planted in wooden flats, was subjected to three treatments: Short day, 9 hours of daylight (one flat); check, about 11.2 to 11.7 hours of daylight (one flat); and long day, 16 hours of light (two flats). The short days were provided by covering the grass with a ventilated light-tight box from 4:30 p.m. to 7:30 a.m. Long days were effected by utilizing a 250-watt Mazda bulb located 3 feet above the grass from 6 p.m. to about 10 p.m. daily. The experiment was initiated November 28, 1944, and concluded March 13, 1945. Results are presented in table 5.

Table 5.—*The production of seed on Zoysia matrella in relation to photoperiodism*

Treatment	Average spikes per square foot			Average fresh weight of seed per square foot		
	Feb. 2	Feb. 20	Mar. 13	Feb. 2	Feb. 20	Mar. 13
	Number	*Number*	*Number*	*Mg.*	*Mg.*	*Mg.*
Short-day	12.0	7.4	40.7	37	18	273
Check	1.2	8.9	19.0	----------	32	110
Long-day	11.6	0	0	21	0	0

Over a period of 3.5 months the short-day light period resulted in the greatest production of seed and the long-day light period the least. The light period of ordinary days, or the check plot, resulted in seed production intermediate in relation to the other two treatments. Seed

collected on the first harvest, February 2, from the long-day plot were probably a result of previous natural exposure to short days since the sod was taken from the station lawn. These spikes were 3 to 4 inches tall, whereas those of the check plot were about 2 inches or less. Spikes from the short-day lot were about 1½ inches in length. The same relationship existed between length of grass blades and light treatment, the long-day blades being about 4 inches high, normal day about 2 inches, and the short-day about 1 inch. Blades from the plot receiving short-day treatment were deep green; those from the long-day plot were a somewhat lighter green and more tender.

Results of this trial are in general agreement with observations of the past several years during which this grass has been grown on the station lawns. The grass normally begins fruiting in October with the advent of short days (11.2 to 11.7 hours) and ceases fruiting in March as the days become longer. In July the days are 13.2 hours.

PHYSIOLOGICAL STUDIES

By David G. White

The area of bark influenced by tapping rubbertrees was measured electrometrically.—The Para rubbertree (Hevea brasiliensis) is the world's main source of natural rubber. The increased demand for rubber in recent years has instigated the use of more intensive tapping systems, which in turn are associated with an increase in brown bast. Brown bast may be described as a physiological degeneration of the bark tissues caused by overtapping. In some plantations there are areas where more than 80 percent of the trees are not in tapping because of brown bast. In many of these cases the reason lies in the use of a two-cut system tapped on alternate fortnights. The two cuts on each tree are placed at an arbitrary distance apart, usually about 30 inches, which has not proved to be sufficient for high-yielding trees; i.e., the lower panels in particular never receive a complete "rest" and as a result develop brown bast. However, no satisfactory method of tracing the area of bark influenced by tapping has yet been developed, although such a measurement would be the basic answer in controlling the incidence of brown bast.

In this study virgin seedling trees with a d. b. h. of 10 to 15 inches (unknown age) had a half circumference cut made 50 inches from the ground. Pairs of terminals 1 inch apart, made from discarded hypodermic needles, were inserted in the bark above and below the tapping cut. An electric current was passed through each pair of terminals by means of a Bouyoucos Bridge (7). Measurements of the resistance offered by the bark between each pair of terminals were made before and after tapping. The resistance increased after tapping much more than possibly could be accounted for by transpiration. This was demonstrated by measurements at intervals throughout the day without having tapped the tree. The increase in resistance following tapping was probably associated with increased concentration of the cell contents caused by the loss of water in the form of latex exuding from the tapping cut. This effect was measurable to a distance of 18 inches below the cut but not at 24 inches. Apparently the area of bark influenced by tapping these low-yielding (about 12 ml. of latex per tapping) seedling trees extended more than 18 and less than 24 inches

below the cut. Therefore, it was assumed that two cuts might safely be placed 2 feet or more apart without causing brown bast on such trees. However, high-yielding clonal trees, which often yield several times as much latex, would no doubt have a much larger area of bark influenced by tapping.

CONTROL OF INSECT PESTS AND DISEASES

INSECT PESTS OF FOOD CROPS

By Harold K. Plank

Rolling seed corn ears in lime protected them from storage insects.—Another experiment was completed in March similar to that reported in 1943 (*21, pp. 21–22*) comparing two organic compounds with hydrated lime in the control of storage insects. USDA–34 sweet corn was again used, but this time the rice moth (*Corcyra cephalonica* (Staint.)) was the only pest noted other than the rice weevil (*Sitophilus oryza*), which occurred in great abundance. The organic compounds were finely ground phenothiazine and a micronized grade of phenoxathiin containing 46.5 percent of bentonite.[11] Rolling the partly dried seed ears in hydrated lime gave practically the same results as before. Insect damage (i.e., infestation of individual kernels) was held to 14.5 percent at 1 month, 34.5 percent at 3 months, and 86.0 percent at 6 months after harvest, all except the last being better by high significance than the untreated grain, in which the corresponding infestation was 61.5, 95.3, and 100 percent, respectively. Germination was over 90 percent at 3 months and 24 percent at 6, both highly significant as compared with the untreated seed, of which 44.3 percent germinated at 3 months and 0.5 percent at 6 months.

Phenothiazine added to hydrated lime at 17.5-percent concentration and applied as above allowed 33.3-percent infestation at 1 month, 45.0-percent at 3, and 79.3-percent at 6 months. Except at 1 month these results were not significantly different statistically from hydrated lime alone, and germination was affected little by the addition of phenothiazine. When phenothiazine was used alone by mixing it with the grain immediately after shelling (1 month after harvest) at the rate of 1 part to 448 parts by weight, the infestation was 88.0 percent at 3 months and 100 percent at 6 months. None of these results was significantly better than the untreated. By either method of application phenothiazine was without practical value in protecting the seed from storage insects.

Phenoxathiin-bentonite mixed with hydrated lime to contain 17.5 percent of phenoxathiin and applied to the ears held the infestation to 26.3 percent at 1 month, 31.5 percent at 3, and 96.0 percent at 6, which, except for the first month, was not significantly different from hydrated lime alone. When the undiluted material was mixed with the shelled grain at the same rate as phenothiazine, the infestation was 42.0 percent at 3 months and 43.8 percent at 6 months; although this material was applied 3 weeks later than the treatments containing lime, the infestation never increased appreciably, with the result that at 6 months it was less by high significance than in any other treatment.

[11] Smith, L. E. phenoxathiin—a promising insecticide. U. S. Bur. Ent. and Plant Quar. E–580, 4 pp. 1942. [Processed.]

From this test it was evident that considerable protection of sweet corn seed from insects and from loss of viability in common storage can be obtained by rolling the seed ears in hydrated lime as soon as the grains separate enough for the dust to penetrate to the cob. Combinations of lime containing 17.5 percent of phenothiazine or phenoxathiin similarly applied were better than mixing either one of these organic compounds alone with the grain after shelling. Between 3 and 6 months after harvest phenoxathiin-bentonite alone applied to the shelled grain held insect damage uniformly lower and resulted in higher germination than any other treatment.

Root caterpillar found severely injuring sugarcane.—In December attention was called to damage to 8-month-old sugarcane showing symptoms resembling severe drought or dry top rot. One grower submitted affected plants in which he had noted injury described by Seín (*27*) as being caused by the sugarcane root caterpillar (*Perforadix sacchari* Seín). Field examinations showed the central spindle of such plants to be wilted or partly dry, as though from attack by the sugarcane borer (*Diatraea saccharalis* (F.)). Another characteristic was an abnormal shortening of the internodes at the top of the stalk, which caused the stalk to have a stunted appearance. Stalks with the above symptoms could be pulled up easily, as though the roots had been severed by the feeding of white grubs. The larger roots of such plants were frequently found to be only a few inches long, with a number of smaller, stubby roots coming out near the end. Often brace roots were sent out at the base of the stalk, and many of these in turn showed characteristic feeding by this insect.

Scattered plants over a number of fields in one plantation near Mayaguez were found with the above symptoms. Spring-plant cane of the POJ 2878 and M 338 varieties seemed to suffer the most and POJ 2947 the least. No symptoms were seen here in M 28 or on any stubble or ratoon cane. Much greater areas of plant cane were found attacked near Hormigueros; one field of about 6 acres of POJ 2878 was heavily infested, with the result that the cane was only about half as tall as normal. Other scattered areas of the same variety were suffering in a similar manner. M 28 nearby was showing typical leaf symptoms, but M 275 was not. POJ 2878 ratoons were also apparently free. Soil moisture in all cases appeared to be adequate.

Although he did not find the place of oviposition in the field, Seín (*27*) reported that he succeeded in securing oviposition in the laboratory beneath bits of roots and trash scattered over moist soil and among the soil particles themselves. It is possible that the rather deep accumulation of leaves and trash from the previous crop still about the base of the stools of the ratoon cane examined at the two foregoing places not only kept the cane in good growing condition through conservation of soil moisture, but also prevented the adult moths of the root caterpillar from laying their eggs in locations where the resulting larvae could develop and injure the roots sufficiently for leaf symptoms to appear at this time. Leaving or placing trash about the base of the cane stool would seem to be indicated as an important method of control.

INSECT PARASITES AND PREDATORS

By Harold K. Plank and Kenneth A. Bartlett

Seven shipments of beneficial insects received from Brazil.—Seven shipments of beneficial insects, mostly parasites of the sugarcane borer (*Diatraea saccharalis*), were received from Brazil through the cooperation of the Bureau of Entomology and Plant Quarantine. One shipment of 250 egg masses of *D. saccharalis* parasitized by *Telenonus alecto* (probably *Prophanurus alecto* Crawford, already established in Puerto Rico) and *Trichogramma* sp. (probably *T. minutus* Riley) came from Campos. From these about 400 adults of *Telenonus* and approximately twice that number of *Trichogramma* were recovered and liberated the next day near Hormigueros. Five shipments from Santa Isabel, in the State of São Paulo, contained a total of 5,776 puparia of 3 fly parasites and 109 cocoons of the hymenopterous parasite *Ipobracon* sp. of *D. saccharalis*. From these there were recovered 1,128 adults of *Paratheresia diatraeae* (Bréthes), 11 *Parthenoleskia parkeri*, 46 *Metagonistylum minense* Towns. (Amazon fly, São Paulo or day-land strain), and 23 of *Ipobracon* sp., all of which after mating were liberated at Hormigueros between February 28 and May 24. The emergence of the fly parasites, particularly *Paratheresia*, was very low, as most of the material was field-collected and heavily infested by hyperparasites; a sample of 1 lot showed over 40 percent of the puparia attacked.

A large shipment of coffee leaves heavily infested with the green scale (*Coccus viridis* (Green)) came from Campos, Brazil. From this shipment there emerged, besides numerous hyperparasites which were destroyed, 1,103 adult females and 296 males of the hymenopterous parasite *Cocophagus heteropneusticus* Compere and 4 females and 1 male of *C. fallax* Compere. One thousand and thirty-five females and 286 males of the former species and 3 females and 1 male of the latter were liberated between January 27 and February 16 near Mayaguez.

Included in this shipment and in another one also originating at Campos were a number of individuals of a coccinellid, *Azya* sp., a predator of the green scale. From these 2 lots numerous hyperparasites of the beetle pupae emerged and were destroyed. The remaining *Azya* beetles, 11 from the first shipment and 12 from the second, were caged February 16 and 25 for reproduction and colonization on green scale infesting gardenia on the station grounds at Mayaguez.

A shipment of ladybeetles, *Chilocorus stigma* (Say), which attack the bamboo scales *Asterolecaniun bambusae* (Bvd.) and *A. miliaris* (Bvd.) was received from Florida through the cooperation of the U. S. Sugar Corporation, at Clewiston. A liberation of 233 of these coccinellids was made at Mayaguez on July 4.

DDT INVESTIGATIONS

By Harold K. Plank

DDT gave promising results against several insects.—An important factor reducing the commercial production of many of the best varieties of mangoes in Puerto Rico is heavy infestation by the West Indian fruitfly (*Anastrepha mombinpraeoptans* Seín).

A preliminary trial in the control of this insect with one and two sprayings of DDT was started in March on trees of the highly susceptible Totafari and Cambodiana varieties. One pound of commercial material was dissolved with slight heating in 1 gallon of soybean oil, and then emulsified with 1 pound of soybean flour in sufficient water, about ½ gallon, to make a slurry. [12] This stock kept well for 2 days at about 28° to 30° C., but by the beginning of the third day fermentation and some separation became evident without, however, impairing quality or interfering with reemulsification. The resulting mixture, about 1.75 gallon, was broken up with water and placed in the spray tank with the agitator running, additional water being added to bring the volume to 100 gallons. A fairly quick-breaking emulsion resulted that spread well over fruit, foliage, and bark. Early results indicated that the infestation in fruit of the Totafari variety was considerably reduced to April 30. Control was variable on the Cambodiana variety and not conclusive at this time.

Some of the above diluted DDT emulsion was used to drench the interior of structures around the station dairy. Approximately 1 gallon of spray was used to each 30 square feet of rough concrete surface and somewhat less for metal, there being considerable runoff in both cases. A few hours after treatment dead insects, mostly stableflies, houseflies, and horn flies were found on the floors and walls. The day following treatment of the milk room, dead cockroaches were seen scattered over the floor. In other buildings, the distribution of light-cardboard tubes smeared on the inside with one-half teaspoonful of the above DDT emulsion stock resulted in considerable kill of cockroaches, particularly *Periplaneta americana* (L.).

BAMBOO PRODUCTION AND INDUSTRIALIZATION

PROPAGATION AND DISTRIBUTION

By David G. White

New industrial species of bamboo received.—Five plants of the Tonkin bamboo, *Arundinaria amabilis* McClure, P. I. No. 110509, from southern China were received during the year through the cooperation of the Division of Plant Introduction of the Bureau of Plant Industry, Soils, and Agricultural Engineering. This species is the principal, and practically the only, bamboo exported from southern China. It produces erect cylindrical culms 20 to 40 feet in length, which are well suited for many uses, of which the most important is in the manufacture of split bamboo fishing rods.

Bamboo species shipped to Latin America.—Considerable quantities of bamboo were shipped to South and Central America through the cooperation of the Office of Foreign Agricultural Relations. F. A. McClure, bamboo specialist for this Office, spent several weeks at the station during May and June arranging for the shipment of this material. A total of 459 culm stumps for planting were sent to cooperative stations at Quito, Ecuador; Bluefields, Nicaragua; and Tingo María, Peru. An earlier shipment of 9 plants was also sent to Nicaragua. The species

[12] Steiner, L. F., Summerland, S. A., Arnold, C. H., and Fahey, J. E. tests of DDT mixtures against codling moth larvae. U. S. Bur. Ent. and Plant Quar. E–628, 17 pp. 1944. [Processed.] (See p. 4, Preparation of spray mixtures.)

distributed were as follows: *Bambusa longispiculata* Gamble ex Brandis; *B. multiplex* (Lour.) Raeusch.; *B. multiplex* var. Alphonse Kaar; *B. textilis* McClure; *B. tulda* Roxb.; *B. tuldoides* Munro; *B. ventricosa* McClure; *Bambusa* sp., P. I. No. 77014, *Cephalostachyum* sp.; *Dendrocalamus asper* (Schultes) Backer; *D. strictus* (Roxb.) Nees; *Gigantochloa verticillata* (Willd.) Munro; *Sinocalamus oldhami* (Munro) McClure; *Phyllostachys bambusoides* Sieb. and Zucc.; and *P. meyeri* McClure.

These shipments represent the first attempts made to disseminate systematically the more important bamboo species which have been introduced into Puerto Rico to other areas in the Western Hemisphere. Dr. McClure also cooperated with the station in the identification of bamboo plantings, and advised on the station bamboo research program.

In addition to the above shipments, a consignment of 10 plants each of 10 different species was sent to Santiago, Dominican Republic, and 368 plants and cuttings of 9 species were sent to La Lima, Honduras, for planting by the United Fruit Co. Two shipments of 170 plants of 4 species were sent to the Department of Agriculture, Jamaica, British West Indies. Thirty-five plants of 7 species were sent to the Imperial College of Tropical Agriculture, Trinidad, and 3 plants of 3 species to the Department of Agriculture, Grenada, B. W. I. One shipment of 6 plants was sent to St. Thomas, U. S. Virgin Islands. Locally, 9,515 plants were distributed through the Soil Conservation Service, to the Army, and to private growers. A total of 29,920 linear feet of cut culms were also furnished to military bases and to the Puerto Rico Development Co.

Two species of bamboo flowered during the year.—The flowering of oriental species of bamboo is generally considered a phenomenon of much interest because it happens at relatively long intervals; 20 to 80 years are often required. Furthermore, the flowering of oriental species is reported to occur simultaneously on all culms and clumps within any clone of a species regardless of age and usually is followed by the death of all the vegetative parts, especially if much seed sets. Thus, large acreages die which may have been producing for many years and the forest must be regenerated from the seed (*2, pp. 95–107*).

Plants of *Bambusa arundinacea* planted at the station 8 years ago flowered and set viable seed during February and March 1945. However, only a few of the clumps and, in several cases, not all the culms in a single clump have flowered. Differences in available soil moisture and fertility were apparently not associated with flowering. The flowering culms did not always die as expected. Even more erratic was the behavior of one plant of *Guadua angustifolia*, now 4 years old. A few of the 1943 culms in this clump flowered in 1944. These culms did not die and, in a few instances, flowered again this season. In addition, several culms produced in 1944 flowered in March 1945.

Roots of bamboo effective in the control of soil erosion.—The soil around bamboo plants is permeated by a mass of intertwining roots. A study was made on a vigorous 8-year-old hillside clump of *Bambusa tulda* to ascertain the distribution of the roots in the soil. From the base of an outermost culm a ditch was dug to a depth of 4 feet for a distance of 17 feet from the culm. The number of roots exposed in each square foot on the face of one wall of the ditch were recorded. Most

of the roots, 83 percent, were present in the upper foot of soil, which is the area where roots serve best in controlling soil erosion. The percentages of roots at lower layers were: 1- to 2-foot depth, 12 percent; 2- to 3-foot depth, 4 percent; and 3- to 4-foot depth, 1 percent. The greatest concentration of roots was within 4 feet of the clump. A few roots were seen penetrating the soil to a depth greater than 4 feet and at a distance of more than 17 feet from the clump.

CURING EXPERIMENTS

By David G. White, Pedro Seguinot Robles, and Milton Cobin

Powder-post beetle infestations varied with curing.—Various-aged culms of *Bambusa tuldoides* were cut during the last week of January 1944 from clumps planted in 1936. Each culm was cut above the second node from the ground so that the stump could be used for propagation. Thus, the basal cuts of all culms were similarly located. Each of the following treatments was applied to five culms of each age as replicates: (1) Check, culms trimmed and stored immediately on horizontal racks under an open shed; (2) clump-cured, culms maintained upright in field clumps for 28 days, then trimmed and stored as in (1); (3) water-cured, culms trimmed and submerged in a pond for periods of 130, 161, and 192 days, after which the culms were removed from the pond and stored as in (1); (4) clump-and-water-cured, culms treated as in (2) for 14 days, then trimmed and submerged for 135 days, after which they were stored as in (1).

The above treatments were applied to culms of four different ages; i. e., current season's culms sprouting in 1943 and approximately 6 months old when harvested, 1942 culms 12 to 18 months old, 1941 culms 24 to 30 months old, and 1940 culms 36 to 42 months old. At the time of storing, each culm was divided into 12-foot sections starting at the base. All tips less than 3 feet in length were discarded.

Results were based on the number of holes made by the bamboo powder-post beetle (*Dinoderus minutus* (F.)) in each internode and distal node in each section of all culms. These observations were made monthly for 6 months during the storage period of each treatment. In some cases heavy infestations prevented exactness, and therefore more than 12 holes in a unit were recorded as 12 plus.

All treatments resulted in less beetle infestation to a highly significant degree than when the culms were merely stored under a shed immediately after cutting. There were no significant differences among other treatments, but, from a practical standpoint, clump curing gave the best results. There was less infestation to a highly significant degree in culms produced in 1940 and 1943 than in those produced in 1941 and 1942. However, culms produced in 1943 had less infestation to a highly significant degree than those of 1940. There were several highly significant differences between treatments within the 1941 and 1942 ages and between ages within each treatment. The basal 12-foot sections had higher infestations to a highly significant degree than the middle 12- to 24-foot sections. The middle sections had higher infestations to a highly significant degree than the tip 24- to 36-foot sections. These facts are in agreement with previous reports (*20, p. 21*).

BAMBOO POWDER-POST BEETLE

By Harold K. Plank

Susceptibility to powder-post beetle attack decreased in older culms.—Culms of *Bambusa vulgaris* Schrad. and *B. tulda* that sprouted in 1939 to 1943, inclusive, were harvested and tested during the spring of 1944 for susceptibility to the powder-post beetle, *Dinoderus minutus*. One culm in each year of growth was used from each of five well-established clumps of each species. Four ¾-inch rings were sawed from an internode from the bottom, middle, and top of the culms. These were used as test pieces and randomized in 2 representative groups of 60 rings each for each pair of clumps and exposed to infestations in cages containing 6 reared beetles per ring. The remainder of the procedure was the same as that followed in previous tests (*21, p. 23*).

Bambusa vulgaris sustained a total of 2,565 beetle attacks, or an average of 8.55 per test ring, whereas *B. tulda* sustained only 375, or an average of 1.25. The first-year growth of both species was attacked the most, but the comparative susceptibility of *B. tulda*, 13.4 percent, at this age was slightly less than the average of the 5 growth years. This first-year susceptibility of *B. tulda* is the highest thus far recorded for this species.

At each age in both species there were more beetle attacks in the test pieces from the bottom internodes than in those from the middle, and more in the middle than the top; in *Bambusa vulgaris* one or more such differences were highly significant, but in *B. tulda* none was significant. The iodine test for starch applied to sample rings from these parts revealed a distribution of starch that in general paralleled the intensity of infestation. Between species there was a marked contrast in reaction to iodine; the samples of *B. vulgaris* showed medium to strong concentration of starch in the wood, but those of *B. tulda* showed little or none. In any one location in either species there was little difference in reaction among the various ages, fifth-year wood showing the presence of nearly as much starch as first-year wood.

Between species at each stage of growth there was a highly significant difference in susceptibility, but among ages of any one species not all such differences were significant. As compared with culms in their first year, the susceptibility in each succeeding age of *Bambusa vulgaris* decreased significantly except between the third and fourth years; it is important from the standpoint of utilization that the number of beetle attacks in the oldest culms of this species was nearly as low as that in the first-year culms of *B. tulda*. In *B. tulda* also, the older culms were all less susceptible than the first-year stock, but only in the second- and fifth-year culms was the difference large enough to be significant. However, from the results of this test, which corroborate those of earlier work (*23, p. 30*), it would appear that much of the damage caused by the bamboo powder-post beetle can be avoided in *B. vulgaris* by harvesting culms in their third year of growth or older and in *B. tulda* by harvesting those in their second year or older.

AGRICULTURAL ENGINEERING

PROPERTY IMPROVEMENTS

By Barton C. Reynolds

In cooperation with the War Emergency Program of the Insular Government the 2,225,000-gallon reinforced concrete water-storage tank project was completed and placed in operation. The site was filled and graded with the surplus excavated earth and the entire area terraced and planted for erosion control. Since this tank is 14 feet in the ground and has an open top, a five-wire barbed fence was placed around the edge which is energized with a standard electric fence controller to ward off animals, rodents, and people. An automatic chlorinating machine was connected to the water distribution mains and provides potable water at all points of use. The completion of these water facilities insures the station of a continuous supply of water throughout the year. Adequate water is now available to all buildings and laboratories with a surplus to be used for emergency irrigation of the experimental fields and plantings during the dry season.

The completion of the large flood-control lagoon constructed in cooperation with the War Emergency Program in the main drainageway through the station grounds has eliminated the hazard of flash flooding of lower laboratories in the new building, and has reclaimed approximately 5 acres of swampland for additional plant introductions. The salvaged area was planted to lawn grass and the drainage channel riprapped with stone.

Air-conditioning equipment was installed in the *Cinchona* greenhouse. Complete automatic control of temperatures is provided as well as the necessary installations for automatic humidity control.

A new location for the library and reading room was prepared and is now in use. A new zinc roof was installed over the area, tile and cement floors placed, and interior walls and ceilings redecorated. A large mahogany framed soils map of Puerto Rico was installed on one of the walls of the reading room, and all furnishings were refinished.

To facilitate the plant introduction program and to insure a permanent record of the field planting locations, an 8- by 12-foot contour map of the main station grounds was prepared to the scale of 1 foot equaling 500 feet. Lettered and numbered coordinates have been superimposed on this map dividing the entire area into sections or blocks. With the installation of permanent markings in the field at the intersection of these coordinates, the exact location of any plant can be permanently recorded. Since the scale of the map is of sufficient size, the plant introduction numbers can also be placed adjacent to the plant locations. One copy of this master map is to be permanently mounted on a wall in one of the offices, and another copy cut into pieces of convenient size for use in the field.

VANILLA

AGRONOMIC STUDIES

By Héctor R. Cibes

Effect of three types of mulch and two degrees of shade on vanilla.—A previous experiment with vanilla (*Vanilla fragrans* (Salisb.) Ames)

showed that better growth was obtained when the vines were planted under one-half and two-thirds shade than under one-third shade (*11*) It was demonstrated in several other experiments that the incidence of vanilla root rot disease (*Fusarium batatatis* var. *vanillae* Tucker) can be significantly reduced when vines are grown in combination with crushed limestone (*28*).

A new experiment was started in March 1944 to compare two types of shade, one-half and two-thirds, with three types of mulch, both alone and mixed with limestone, and to establish the degree of interaction between mulch and shade on the production, growth, and disease resistance of the vanilla vine. The mulches used were obtained from three different soil types, namely, Catalina clay (pH 5.0), Toa clay (pH 6.3), and Soller clay (pH 7.8).

The experiment is located on soil of the Catalina series. A lath house provides the shade with its longer sides having an east to west exposure. The different shade intensities were achieved by spacing split bamboo at proper intervals both on the sides as well as the top. Drainage of the beds is provided by 3 to 4 inches of coarse gravel beneath the mulch and between plots by ditches constructed between shade plots and between every three beds. Small living bucare cuttings are planted along the sides of beds for holding the mulch in place. The experiment was divided into 8 plots, each plot consisting of 2 shade tratments. Within each shade treatment a set of 6 mulch treatments was randomized with 4 vanilla plants per treatment making a total of 96 replicates.

The vanilla plant derives a large portion of its nutrient requirements from the mulch in which it grows. Composite samples of each type of mulch were taken from two applications made during the course of the year and analyzed for phosphorus, potassium, calcium, magnesium, and nitrogen.[13] Table 6 shows the amount of these elements present in the various types of mulches.

TABLE 6.—*Analyses of composite samples of fresh mulch obtained from two successive applications of mulch from Catalina, Toa, and Soller soils* [1]

Element	Mulch [2]	Types of mulch		
		Catalina	Soller	Toa
Total ash	1	7.61	11.85	17.68
	2	8.97	7.97	31.42
Total N	1	.67	1.14	1.34
	2	.97	.72	1.98
K	1	.39	.20	.71
	2	.25	.11	.25
P	1	.03	.08	.21
	2	.07	.05	.28
Ca	1	.47	1.20	.69
	2	.75	.33	.94
Mg	1	.10	.85	1.10
	2	.55	.10	2.80

[1] Expressed on the basis of percent of dry weight of material.
[2] Mulch 1 was applied at the beginning of the experiment in March 1944; mulch 2 was applied about 7 months later.

[13] Analyses were made by Gilda C. Vicente.

In a previous experiment vanilla leaves from different locations were analyzed (*28*). It was found that the percentage of calcium in the leaves varied widely in relation to the amount of this element present in the soil. However, there was but little variation in the quantity of other elements. As pointed out in this previous work, the calcium content of the soil was apparently associated with the incidence of root rot disease.

In making comparisons between mulch 1 and mulch 2 it will be noted that Soller mulch surpassed all the other mulches in calcium content and some other elements in the first analyses, but it ranked last in almost all elements in the second mulch application. This sharply contrasting difference in chemical composition of Soller mulch was probably due to the fact that mulch for the second application was obtained from a different site, and different plants may have been involved as well as slightly different soil conditions. It remains to be seen if this variation in mulch composition will affect the ultimate growth of the respective plants.

Significant differences in growth found among mulch treatments.—The first data secured at 6 months on the vegetative growth made by vanilla plants under the various treatments brought out some contrasting differences between treatments, but there were few significant differences between treatments at this stage of growth. The vanilla plants growing in mulch obtained from Catalina clay either with or without limestone were inferior in growth to all others. There was little or no difference in growth in plants growing on mulch obtained from Soller and Toa clay between shade treatments.

The second measurements of the amount of vegetative growth made by vanilla plants grown under different mulch and light treatments were taken in March 1945, one year after planting. Covariance analyses of the growth data showed that there existed highly significant differences between the various mulch treatments. The differences required for significance at both light levels were greater than the actual F value for mulch. This indicated that the mulch treatments were the factors which exerted the predominating influence upon growth of vanilla irrespective of shade treatment. The significance between individual mulch treatments was also calculated. This is shown in table 7 for comparison of the six treatments. The standard error of the difference for the mean is 17.06. Differences between mulch treatments to be highly significant on the 1-percent level must be greater

TABLE 7.—*Significance of differences in vanilla growth produced on the various mulch plots*

Treatment [1]	Mean yield [2]	Significance
F	932	Better than A, C, D, E.
B	928	Better than E, C, A, D.
E	884	Better than D, A.
C	871	Better than D, A.
A	738	
D	712	

[1] A = Mulch from Catalina soil.
B = Mulch from Toa soil.
C = Mulch from Soller soil.
D = Mulch from Catalina with limestone.
E = Mulch from Toa with limestone.
F = Mulch from Soller with limestone.

[2] Differences necessary for significance at 5-percent level—33.95 inches; high significance at 1-percent level—45.21 inches.

than 45.21 and on the 5-percent level greater than 33.95. Soller mulch mixed with limestone (F) produced better growth by high significance as compared with the other treatments, excepting Toa mulch used alone (B). The poorest growth under all treatments was made with treatment D, which was Catalina mulch mixed with limestone.

Data taken on aerial root formation and girth of vanilla vines at the sixth node from the tip were not significantly influenced by the light treatments. There were, however, some contrasting differences between aerial root growth made by plants growing in the various mulches. Vines planted in Catalina mulch alone or mixed with limestone produced the least number of aerial roots, 208 and 206, respectively, as compared with 248 and 234 for Toa clay and 227 and 234 for Soller. The smallest average girth measurements were 3.89 and 4.10 cm., respectively, for plants growing in Calatina mulch with or without limestone, as compared with 4.46 and 4.48 cm. in Toa and 4.45 and 4.55 cm. in Soller.

CHEMISTRY OF VANILLA PROCESSING

By Gilda C. Vicente and Merriam A. Jones

Vanilla curing involves nonenzymatic as well as enzymatic changes.— The term "curing" is commonly used to denote the over-all changes that take place during the processing of many different types of raw material. It includes the preparation of such diverse products as vanilla, tobacco, cheese, whiskey, and derris root. The actual changes that occur in all of these curing processes can be classified as those involving (1) no chemical transformations, that is, changes involving only the loss of water, and (2) changes principally in the chemical composition in which loss of water is only of minor importance. The first class, including such processes as curing of derris root and probably that of ginger, involves primarily drying; any chemical changes that do occur may be deleterious to the product. The second class includes curing of such items as cheese and vanilla in which hydrolytic and oxidative changes are of more importance than dehydration alone. This class can be further subdivided into curing processes in which the chemical changes are brought about principally by (1) enzymes, as in the hydrolytic cleavage of glucovanillin in vanilla, and (2) simple chemical transformation without enzymes, such as in whisky curing. Those processes depending upon enzymatic action can be further classified according to the source of the enzyme: Foreign enzymes, as those furnished by micro-organisms in the curing of cheese, and intracellular enzymes, those contained in the material to be cured.

Although curing processes can be thus classified for the purpose of clarification of thought, many are not entirely of one type. For example, vanilla curing, although commonly considered to depend upon enzymes, may, at least in certain phases, be nonenzymatic. Last year it was shown that during the early stages of curing, enzymatic oxidation occurred (*29*), and it has long been known that the cleavage of glucovanillin to glucose and vanillin is brought about by a hydrolytic enzyme. The importance of enzymes in the later stages of curing was not evaluated. Therefore, in a recent experiment beans were autoclaved at various stages of the curing process to see what kind of product would result. The autoclaving at 120° C. was calculated to kill completely

the enzyme system so that subsequent changes could not be considered enzymatic in nature. However, it was found that, although oxidase, catalase, and peroxidase were inactivated by the autoclaving, peroxidase later became active again at least to a certain extent. It was also found that oxidase, as well as peroxidase, "came back." This was shown by autoclaving two samples of cut beans, one of which was kept sealed while the other was exposed to air but not enough to allow it to dry. After 2 weeks pieces exposed to air began to brown but sealed ones remained green. After 6 months the exposed sample had a weak, sweetish aroma, not like vanilla, whereas the sealed sample was still green. Therefore, although the rejuvenation of oxidase could not be shown by a direct measure of oxidase activity, it must have actually occurred.

For the experiment in inactivation during curing, vanilla beans were killed in hot water and then sweated, dried, and conditioned. Samples were autoclaved at the following stages: Immediately after killing, 2 days after killing, at the end of the sweating process, and at the beginning of conditioning. The controls became dark brown and developed vanilla aroma. Before killing some catalase activity was found, but after killing none could be shown. Likewise oxidase, although it is known to be important, was not demonstrable by direct measurement after killing. A high peroxidase activity was noted in the fresh beans. If the peroxidase activity of fresh beans is taken as 100 percent, the activity in the controls rose to 115 after killing, to 133 two days later, dropped to 94 after sweating, and rose to 270 at the beginning of conditioning. After conditioning for 2 months the activity was 400 percent of that of the fresh beans.

The samples autoclaved after killing stayed green for several weeks and finally became greenish yellow. The beans took on a burnt fermented aroma not at all like the aroma of vanilla. As to peroxidase, the activity dropped to 19 percent but rose to 28 at the beginning of conditioning. The beans autoclaved 2 days after killing were light brown and less disagreeable in aroma than those autoclaved immediately after killing but did not develop a vanilla aroma. Some mold developed but was eradicated.[14] The peroxidase activity dropped to 9 percent of the original value upon autoclaving and then recovered to 16 at 43 days, and 12 percent at 3 months. The beans autoclaved 1 month after killing approached the controls in quality; they became dark brown and developed a vanilla aroma which was as strong as that of the controls. The peroxidase activity dropped to 23 after autoclaving and to 13 after 2 months' conditioning.

From these results it appeared that the principal changes brought about by enzymes occurred during the first 2 weeks of curing. Enzymatic transformations take place during killing but gradually become less important until, by the end of the sweating period, nonenzymatic changes are dominant. The fact that peroxidase, after being inactivated in the autoclave, recovered to about the same extent in all of the treatments indicated that this enzyme was not responsible for changes subsequent to sweating. This was also indicated by the fact that although the

[14] "Mycoban" (sodium propionate), either in saturated alcoholic solution or as a powder, has been found to be rather effective in eradicating mold on vanilla.

peroxidase activity was exceedingly high in the controls and comparatively low in those autoclaved 12 and 43 days after killing, all three treatments resulted in about the same product. Presumably then, since the enzymatic changes take place during the first few days of curing and since the principal development of vanilla aroma occurs during the conditioning, a considerable part of the curing process must be nonenzymatic.

Oxidative changes during curing tend to bring out a prunelike aroma.—On the basis of the results of the foregoing experiment the role of oxygen in the curing process was investigated. It was shown last year [29] that the principal oxidizing reactions of the vanilla enzyme system were brought about by an oxidase. The present experiment was designed to show the effect of aeration on the bean tissue during curing by cutting the whole beans in various-sized pieces. A second objective was to determine whether such a method could be used to simplify the curing methods commonly used.

The procedure was to prepare the beans by five methods: (1) whole, (2) cut to 1-cm. slices, (3) ground through a food chopper, (4) ground with a pestle with sand in a mortar, and (5) sectioned longitudinally into one sample of the pod wall and one of the placental tissue and seeds. The difference between treatments (3) and (4) was that, in the former, few cells would be broken but in the latter most cells would be ruptured. The samples were then oven-killed at 60° for 24 hours, sweated until the whole beans were flexible, dried to 28.6 percent of the fresh weight, and conditioned in closed jars.

The whole beans became dark brown and developed a suave, flowery, vanilla aroma. The beans cut to 1-cm. slices became dark brown and had a prunelike aroma; vanilla aroma was good but not so good as that of the controls. Samples ground in the food chopper turned brown on top where exposed to air but became dark brown throughout only after mixing. The aroma was like that of the cut beans except that the prunelike note was even stronger, in fact, so strong that no vanilla aroma could be detected. Beans crushed in the mortar also turned brown on top where exposed to air and had to be mixed to obtain a uniformly dark-brown mass. While drying, a slight vanilla aroma developed somewhat like that of the controls but not so strong. The seed and placental tissue developed no aromatic character. The pods without seeds and placental tissue cured brown and developed a strong sweet aroma with some vanilla character. During conditioning a fermented acid note became noticeable but vanilla aroma was still present.

It was concluded from this experiment that excessive aeration such as obtained with cut or coarsely ground beans resulted in excessive oxidation and a consequent development of a prunelike note in the aroma. Less contact with air as in whole or finely crushed beans resulted in better vanilla aroma. Therefore, curing of cut or coarsely ground beans must be carried out with less contact with air in order to produce a good quality vanilla aroma.

Conditioning vanilla above room temperature gave a superior product.—Previous experiments in vanilla curing have involved different methods of killing, sweating, and drying with only one method of conditioning. Since the main development of flavor takes place during conditioning, an investigation of this phase was made.

Three lots of beans were frozen solid overnight at −10° C., thawed, and killed in hot water. After sweating and drying, conditioning was carried out in closed cans using three temperatures, 13°, 27°, and 35°. During the curing process no mold developed on any of the samples and no color differences were apparent. The aroma of the warm-conditioned beans developed more fully than those conditioned at room temperature or at 13° and had a pungent note characteristic of Mexican vanilla. Those kept at room temperature were characterized by a more flowery aroma typical of Puerto Rican vanilla and eventually the aroma was much more developed than that of those conditioned at 13°. The first vanilla crystals appeared on the beans kept at low temperature after 6 months' conditioning. Those at room temperature had crystals less than 10 days later, but those at 35° produced no crystals.

After conditioning for 6 months organoleptic tests showed that the seeds in all three lots were almost tasteless and sandy. All of the flavor was in the outer wall. Those kept at the lowest temperature were the most pleasant, and, as the temperature of conditioning was higher, the beans became more bitter. The outer wall of those kept at 35° was bitter and had a taste remindful of chewing tobacco. After 7½ months of conditioning, moisture determinations showed that those beans kept at low temperature contained 27.9 percent of moisture, those kept at room temperature 21.9, and those kept in the oven 15.8 percent. Extracts were prepared with 10 ml. of 50-percent alcohol per gram of dry vanilla by chopping the beans to 1-cm. slices and soaking in alcohol in a flask fitted with an air condenser. Over a period of 2 weeks the flasks were heated twice daily in a water bath to 60° after which the extracts were strained through cotton to remove solids. Vanillin analysis by the A. O. A. C. method (*5, pp. 320-321*) showed that all the samples contained about 3.5 percent vanillin on the dry basis and no differences existed among treatments.

During the past season this experiment was repeated with the following modifications: The samples were killed in hot water at 80°, kept in blankets for 1 day, and then oven-sweated in blankets, dried, and conditioned using four temperatures, 45°, 35°, 27°, and 13°.

After conditioning for 6 months it was found that those at 45° had the strongest vanilla aroma accompanied by a sweet prunelike note. At 35° the product was not so strong but was more suave. Those kept at 27° and at 13° were comparatively poor. Apparently high-temperature conditioning brought out background fixative qualities not so noticeable in the ordinary cured material. It is also important to note that the beans at 45° were completely cured after about 3 months of conditioning. Thus far the results of this experiment corroborate those previously discussed.

A technique for testing vanilla by means of ice cream tried.—In general it is agreed that the criteria usually applied to vanilla do not constitute a good measure of quality of the product. Vanillin analyses, phenol value, lead number, and resin content are of little value in themselves. Even the usual grader's examination for texture, color, aroma, and appearance, although of value for rapid estimation of quality, is not entirely satisfactory. However, all of these criteria have some value when taken together, but, especially when differences among samples

are small, they do not constitute an entirely satisfactory measure of quality.

Vanilla as well as other similar materials can be tested organoleptically by several testers and their judgments considered statistically to evaluate the differences among the samples. This method was tried on the extracts from the foregoing conditioning experiment in which three temperatures were used, both to evaluate the products and to try the technique of testing (*6*). These same extracts were used numerous times at various strengths and in different recipes. The most satisfactory recipe to date has been 1 pint of cream (40-percent butterfat), 4 pints of milk (4-percent butterfat), 373 grams of sugar, and 7.5 grams of gum tragacanth. The mix was divided into separate portions for each sample, and the extract added at the rate of 2 ml. per 100 ml. of mix. After cooling to 10° the samples were churned in a hand freezer to a hard consistency and stored at −10° until tested.

The ice cream samples were tested from 3 to 22 hours after freezing in two series with coded designations. The ranking by each tester was checked for reliability by calculating the correlation coefficient between the two separate rankings made by the same tester. The data were accepted as reliable if the tester obtained the same ranking both times, or, if in the second run, he interchanged two samples other than the blank that were consecutive in the first run. However, if the tester interchanged first and third rank, the correlation coefficient, then below 0.77 was considered too low, and the tester's data rejected. From the ranks obtained by reliable testers, the data were then treated statistically as though each ranking were made by a different tester. For example, if three reliable testers made two runs each, the final statistical analysis was made as though there were six testers. The same set of extracts was used on several different dates, and the scores for the samples were calculated. If on any date the differences between scores were not considerably greater than that necessary for high significance, the data for that date were discarded on the reasoning that the recipe used was not such that the differences between samples were brought out sharply. Of course, if this occurred consistently the data would be accepted and the conclusion would be that the differences among the samples were not great. However, where great differences were obtainable on some dates, such as was the case with these samples, the runs in which differences were small could be discarded and the weakness of the data attributed to the recipe.

Four runs were made with these samples but in two the scores, although different by high significance, were not greatly different. Whether or not the ranking in these two runs was discarded, the order of decreasing quality was (1) warm-conditioned, (2) room-temperature-conditioned, and (3) cold-conditioned. Using only the two runs in which the scores were very different, the data showed that the beans conditioned in the oven were better than those kept at room temperature, but the difference in scores was not significant. However, between both of these samples and those conditioned at low temperature, the difference in scores was highly significant. The differences between all samples and the blank were also highly significant.

Scratching was the best method for killing vanilla.—In 1943 an experiment comparing different curing methods showed that scratching vanilla beans with a pin was an excellent method for killing (*1*). This year further trials were made with combinations of the following killing methods by pairs: Dipped in water at 80° C. three times for 10 seconds at 30-second intervals; scratched on each face 1 mm. deep from end to end, with some scratched only to 2 cm. from the stem end; frozen at —10° for 24 hours. After killing, the samples were oven-sweated, dried, and conditioned. No mold developed on any of the samples, and splitting was negligible.

Those killed with hot water were dark brown, somewhat rough and not flaccid; no vanillin crystallized. The aroma was prunelike with a strong vanilla character.

Beans killed by scratching required about 1 day to become brown, which was somewhat longer than other treatments. Those scratched to 2 cm. from the stem end had a firm lump in this position, which later disappeared and left the stem end flexible; those scratched along the entire length became woody at the stem end. The beans were dark brown with some reddish cast after conditioning. The aroma was strong, flowery, very agreeable, and fully developed. Scratched beans were the only ones on which vanillin crystallized. It was also noted that these beans were completely cured in about 2 months. The combination treatment consisting of a hot-water kill followed by scratching, as well as that consisting of scratching followed by a hot-water kill, resulted in a product similar to that of the hot-water kill alone. However, the vanilla aroma was not so strong.

The samples killed by freezing were characterized by a reddish-brown color and a very flaccid texture. The product had a sweet, suave aroma, with less vanilla character than those killed by the hot-water or scratching process. The combination treatments hot water-freeze, freeze-hot water, scratch-freeze, and freeze-scratch resulted in a product like that from frozen beans. The color was reddish brown and the aroma sweet. Of the combinations the scratched-frozen beans had the strongest vanilla aroma.

The three single killing processes ranked as follows in descending order: Scratching, hot water, freezing. The scratching method was best when the scratch was not carried all the way to the stem end. None of the combination procedures were outstanding improvements over the single methods.

Inactivated vanilla infiltrated with crude enzymes.—It is well known that an enzymatic step is involved in the hydrolysis of glucovanillin and in some of the oxidative changes that result in browning. In order to learn more about the general nature of the enzyme system that acts in the curing process the following technique was used: Cut beans were autoclaved to inactivate the natural enzymes and were then infiltrated with crude enzyme extracts prepared from other materials containing enzymes of a known nature.

For this purpose vanilla beans cut to 5-mm. slices were autoclaved for 5 minutes at 120°. An untreated sample as a control was oven-killed and sweated until the pieces lost turgidity and became brown. After drying the control lot was conditioned in a closed jar. Pieces

to be treated were covered with the extract to be infiltrated and placed in a vacuum chamber. Upon evacuation to 4 cm. pressure, the air in the cells of the plant tissue bubbled out. The vacuum was released about 5 minutes after bubbling ceased, and, as air entered the chamber, the cells filled up with the extract in which the pieces were submerged. The infiltrated pieces were then drained and cured in the same manner as the control.

The enzymes used and their activities are given in table 8.

TABLE 8.—*Activities of enzyme preparations used for vacuum infiltration of vanilla*

Source	Peroxidase	Oxidase
Vanilla	Medium	High.
Yeast	None	None.
Emulsin	None	None.
Mushroom	Low	High.
Turnip	High	None.

The vanilla samples were infiltrated with extracts representing a like amount of the other plant materials. This experiment was carried out twice because, in the first run, oxidation took place in the autoclaved samples and also considerable mold appeared. However, the results were similar in both trials.

It was clear from the results that, after the natural vanilla enzymes were inactivated, infiltration with an oxidase preparation gave a product that resembled the ordinary cured vanilla. The other enzymes such as preoxidase, alpha-glucosidase, and beta-glucosidase gave products with no vanilla aroma. This confirmed the conclusion that an oxidase was responsible for most of the oxidizing action in the development of vanilla aroma that occurs during curing.

ESSENTIAL OILS

AGRONOMIC STUDIES

By Pedro Seguinot Robles, Noemí G. Arrillaga, and Merriam A. Jones

Optimum height for cutting lemon grass depended on variety and age.—It is generally recommended that lemon grass (*Cymbopogon citratus* (DC.) Stapf.) be cut 5 to 9 months after planting and three or four times a year thereafter. According to de Jong, the percentage of oil in the grass decreases with age while the percentage of citral in the oil increases (*18, p. 72*). In a previous experiment it was shown that the percentage of oil in the grass was greatest at the age of 12 to 15 weeks, but as the interval between harvests was increased from 12 to 15, 18, and 21 weeks, the total amount of oil and citral produced per unit area per unit time increased (*25, pp. 25–26*). However, yields varied considerably because of differences in the amount of growth between harvests, which were brought about by changes in rainfall and other conditions. This experiment is now being supplemented by a height-at-harvest experiment in which the time of cutting is determined by the height to which the grass has grown rather than by arbitrary time intervals.

In this experiment Java and the West Indian varieties of lemon grass were harvested whenever the three replicated plots per treatment attained the heights of 2 feet, 2½ feet, and the maximum growth for the variety. Maximum growth was considered to be the height at which no measureable growth took place during 4 successive weeks (about 4 feet). Monthly applications of fertilizer made from August to November 1943 and from April to November 1944 consisted of a 6–9–10 formula at 1,000 pounds per acre per year.

The data obtained for the first harvest showed that, although the Java variety yielded more grass than the West Indian, the oil and citral yields per acre were about the same. In the second and third cycle of harvests the Java variety yielded considerably more grass and oil, and, although the percentage of oil and citral was less, the total was greater per acre than for the West Indian. As to the optimum height for harvest of the Java variety, the greatest yield of oil and of citral per acre in the first and third harvests was obtained from the grass cut at maximum height. In the second harvest the plots cut at 2½ feet gave the most oil and those cut at 2 feet gave more than those cut at maximum height, but the severe drought unquestionably affected these results. For the West Indian variety the first harvest at maximum height gave more grass but about the same amount of oil and citral per acre as the harvest at 2½ feet. Cutting at 2 feet resulted in low yields. In the second harvest the yields of both varieties at 2½ feet were considerably greater than those at 2 feet and at maximum height. In the third harvest the yields of both varieties at 2 feet and at maximum height were about the same and greater than 2½ feet. Until additional harvests are made no definite conclusions can be drawn.

Ash analyses of the lemon grass showed that the mineral content was low, P_2O_5 being about 0.1 percent on a dry basis; CaO, 0.8; K_2O, 0.3; and MgO, 0.3. Calculations of the amount of minerals removed by the grass indicated that, with the fertilization used in this experiment, none of these four elements would constitute a limiting factor.

Guatemala and Java varieties of citronella grass gave about equal yields.—An experiment similar to the foregoing was started with the Guatemala and the Java varieties of citronella grass (*Cymbopogon nardus* (L.) Rendle), with the modification that the heights at which the grass was harvested were 3½ feet, 4½ feet, and maximum growth (about 6 feet). The data showed little difference between varieties. In the first cycle of harvests the results at 4½ feet and maximum height were about the same with respect to yields of grass and of oil per acre but the harvest at 3½ feet yielded considerably less. In the second harvest the yields at 3½ feet and at maximum growth were higher than the yields at 4½ feet. This was probably due to the replacement of stools that died because of the drought.

The percentages of the minerals in the citronella grass were approximately the same as in lemon grass. Because of the larger yield of grass the amount of minerals actually removed from the soil was considerably higher than for lemon grass.

High-quality bay leaves found in low rainfall areas.—A survey of the bay-rum trees (*Pimenta racemosa* (Mill.) J. W. Moore) established in different sections of the island is being conducted to study some of

the factors which affect the percentage of oil in the leaves and the quality of the oil. Another objective is to find outstanding trees to be used as a source of propagating material.

The influence of the following factors on the oil content of the leaves is being studied: (1) The location of the planting, (2) season of the year, (3) the age of the tree, (4) position of the leaves, (5) maturity of the leaves, and (6) the shape of the leaves. To date 73 samples have been collected and analyzed for oil content and quality. Although the population at present is small, some indications are apparent. The data in table 9 show the variation in the oil content of leaves from different sections.

TABLE 9.—*Oil content of bay leaves from different regions in Puerto Rico*

Municipality	Samples	Oil content of leaves		Annual Rainfall [1]
		Range	Mean	
	Number	*Percent*	*Percent*	*Inches*
Adjuntas	19	1.03–1.84	1.6	88.4
Mayaguez	17	1.11–2.12	1.6	81.4
Ponce	14	1.19–2.22	1.7	80.0
Guayama	16	1.80–2.19	1.9	65.0
Cabo Rojo	8	1.40–2.76	2.1	49.0
Summary		1.03–2.76	1.7	

[1] Data obtained at nearest rainfall gage.

The lowest oil content was obtained at Adjuntas. All of the bay plantings tested so far are on Mucara soils except the one at Mayaguez (Las Mesas), which is on Nipe clay. Root development on the Nipe clay is somewhat limited by underlying rock, and, although the trees are 7 years old, they have not attained normal size. The leaves from Cabo Rojo were definitely superior in oil content to those from the other districts, but a factor to be considered is that these trees have not been harvested for at least 3 years. Leaves from Guayama had the second highest oil content, and the samples collected had the narrowest range of oil content of the group. Generally the regeneration of leaves in this area is fast, and the yields of leaves are said to be high. There appeared to be an inverse relationship between oil content and rainfall. However, since other factors may have contributed to these results, no definite conclusion can be reached at present. Additional data showed that leaf samples taken from the upper and lower parts of the tree were about equal in oil content. The phenol content for all of these samples was high, because of the fact that the distillate was ether-extracted to obtain exact yields, thus the emulsified and dissolved material in the distillate water was recovered. The latter consists mostly of phenols, making the total phenols higher.

Fresh bay seed readily germinated.—Ripe fruits of the bay-rum tree tree were washed in tap water to separate the seeds from the pulp, after which the seeds were spread on filter paper to dry. Each day for 10 days a lot of 50 seeds was placed on damp cotton in a covered petri dish for observation. Seed was considered germinated when the hypocotyl broke through the seed coat. This usually started in 3 days. The viability of the seeds planted on cotton 1 day after harvest was 98 per-

cent; 2 days, 68 percent; 3 days, 98 percent, but on the fourth day the germination dropped to 10 percent. After the fifth day no germination was obtained. It was concluded from this preliminary study that, since the natural germination period of bay seed was extremely short, the seed should be planted immediately after harvest.

Aerodefoliant gave poor results with bay leaves.—The possibility of utilizing a gas such as ethylene to induce abscission of essential-oil-bearing plant parts to facilitate their harvest was reported upon several years ago (*4*). Recently a powder was tried as a defoliant on the bay-rum tree *P. racemosa*. This material, called Aerodefoliant, was calcium cyanamide and has been used extensively to defoliate the cotton plant so that the bolls ripen evenly and can be harvested with mechanical pickers. In using this defoliant on bay leaves, the powder was dusted with an air gun on dry as well as wet leaves. The rate of application varied from being practically invisible on the surface to complete coverage so that some leaves were quite black. No effect appeared immediately, but after several days many leaves were burned to a dead brown and began to abscise. However, since the dropping was slow and the leaves which dropped were weak in aroma, the procedure was deemed impractical as a method of harvesting.

Fungicide treatment of ginger seed pieces did not increase stand.—In previous plantings of Chinese ginger (*Zingiber officinale* Rosc.) comparatively low stands of about 60 percent were obtained, even though the sets were planted with sprouted buds. Hence, an experiment was made to test various commercial brands of fungicides as seed treatments that might decrease rot. The following fungicides were used: Arasan, Cuprocide, Semesan, Spergon, Thiosan, and Wettable Spergon. These fungicides were applied at the rate of 4 ounces to each 100 pounds of seed for the powdered forms and at the rate of 0.5 percent in water for the Wettable Spergon. Dust applications were made in the field by putting the seed pieces in a large paper bag and shaking with the powder, and wet applications by dipping in the solution. The planting was made on March 22, 1944, in rows of 75 plants 1 foot apart, with a border plant at each end. The rows were 2 feet apart.

It was observed that slow germination occurred in spite of daily, but not excessive, watering. Some of the seed pieces rotted even after the plants had broken through the soil. The results showed no differences between treatments. It was concluded that such treatments at planting time were not effective in increasing the stand of ginger.

PROCESSING STUDIES

By Noemí G. Arrillaga and Merriam A. Jones

Solvent extraction gave highest recovery of essential oils from water.—The recovery of essential oils by steam distillation utilizes a considerable amount of water varying from 100 to 500 volumes per volume of oil according to the nature of the oil. Unless an efficient separator is used or the water is cohobated a great amount of oil is lost because of being in either solution or emulsion.

To give an idea of the magnitude of these losses three oils of different characteristics were studied: (1) Lemon grass oil, lighter than water

and containng an unstable aldehyde, citral; (2) citronella oil, also lighter than water, but consisting of relatively stable constituents; and (3) bay oil, consisting of two fractions, one heavier and one lighter than water, a phenol and a terpene, respectively. Two methods were used to prepare intimate mixtures of a known weight of oil in a large volume of water. The first method was to distil oil from water and collect the whole distillate with no provision being made for gravity separation. The second method was to add the oil to the water in a large bottle and shake violently. The oil was then recovered from these mixtures by three methods: The mixture was poured slowly through a Florentine flask and the oil recovered by gravity separation; the other two methods consisted of solvent extraction three times with two different solvents, ethyl ether and petroleum ether, using a giant separatory funnel (*15*). The solvent was then removed by distillation in a water bath at 55° C., after which the last traces were removed by suction applied to the flask while it was swirled in a water bath. Appropriate analyses were made of the oils before and after treatment.

The data obtained showed that in general a considerably greater proportion of oil was recovered by solvent extraction than by gravity separation. If these large losses of oil are to be avoided, a much more efficient gravity separator than a simple Florentine flask must be used. Comparison of the two solvents used for solvent extraction showed that they were about equal. Poor recovery was effected with the lemon grass oil in which the mixture was made by distillation. This was undoubtedly due to oxidation of the citral, which rendered it nonvolatile. Less than 55 percent of the total oil and less than 36 percent of the total citral could be steam-distilled. When the mixture was made by shaking the oil in water, a thick emulsion was formed which resulted in less oil being recovered by gravity separation. With this method of mixing, nearly all of the oil and citral was recovered by solvent extraction. With bay oil, gravity separation was poor, owing to the formation of a heavy emulsion of oil in water. With citronella oil, considerably more oil was recovered by gravity separation, when the mixture was made by distillation. Comparison of the two methods of mixing showed that about two-thirds of the oil lost was lost because it would not distil.

In laboratory work on essential-oil-bearing plant material, it is clear that simple gravity separation is a poor method for obtaining the yield of oil; either petroleum ether or ethyl ether should be used. In commercial practice, the need for cohobation of the water is emphasized by this experiment. It is probable, though, that in most cohabation arrangements some of the oil becomes polymerized or is exposed to oxidation and is then lost when put back into the still pot. That such is the case is indicated by the fact that laboratory distillation, involving final extraction of the oil with ether, invariably gives higher yields than corresponding commercial distillations. Perhaps more complete recovery on a commercial scale could be brought about by improving separators or using some type of continuous liquid-liquid extractor.

Steel treatments failed to prevent iron reactions with essential oils.—Essential oils are usually stored and shipped in galvanized steel or tinned containers. Experimental work to observe the effect of metals

on essential oils has shown that tin is the best metal to be used in contact with the oils because it does not change the physical and chemical properties of the oil. Copper is occasionally used, because it is more readily available and is cheaper in peacetime, but if used it should be tin-plated. It is well known that iron cannot be used in contact with essential oils. At present, with tin and zinc scarce because of war conditions, a method is needed for treating ordinary steel drums to render them suitable for shipping essential oils.

A method involving the treatment of steel was tried to see whether passive iron would react with essential oils. Pieces ½ by ½ inch of an ordinary kerosene drum were cleaned with acetone, alcohol, and soap and water, after which some were rendered passive by the following methods: (1) Immersion in nitric acid (sp. gr. 1.45); (2) immersion in nitric acid (sp. gr. 1.35), stopping the reaction by touching the metal under the acid with a piece of platinum; (3) rinsing with concentrated hydrogen peroxide; (4) using iron as the anode in sulfuric acid electrolysis; (5) bluing, i. e., after cleaning with $K_2Cr_2O_7$–H_2SO_4, washing with ammonium hydroxide, and rubbing dry, ammonium polysulfide was applied and the piece dried, rubbed, and finally rubbed with linseed oil.

Pieces of metal rendered passive by the above methods as well as untreated pieces were immersed in bay, citronella, and lemon grass oils. Bay oil was affected by all the treatments almost immediately except the one treated with hydrogen peroxide, in which case no deleterious darkening of the oil was noticeable until 1 week had passed. Lemon grass oil took longer to react, and citronella oil was the least affected. However, after 3 weeks all the oils had changed in color, appearance, and aroma. The same results were obtained in a supplementary test carried out to check the action of strong nitric acid (sp. gr. 1.45) and hydrogen peroxide to which small amounts of acid or base were added.

From this experiment it can be concluded that, inasmuch as all of the treatments resulted in degradation of the oils, none can be recommended.

COFFEE

VARIETY TRIALS

By Luis A. Gómez[15] and José Lería Esmoris[15]

Columnaris coffee continued to outyield the Puerto Rico variety.—Yields of the Columnaris variety of *Coffea arabica* L., from Java, and the West Indian variety were compared for the eleventh crop year during 1944. The Columnaris continued to be the superior yielding variety. Yields of both varieties in pounds of marketable coffee per acre were: Columnaris 1,125 pounds and West Indian 881. The average per acre yields for 11 harvests were Columnaris 1,166.1 pounds and West Indian 668.

[15] Agricultural Experiment Station, University of Puerto Rico.

SOIL CONSERVATION

OBSERVATIONAL INVESTIGATIONS

By U. S. Allison,[16] Emery A. Telford,[16] Louis B. Earle,[16] and Norman F. Childers

Tropical kudzu shows promise as a cover crop.—Tropical kudzu (*Pueraria phaseoloides* Benth.) is the best perennial, mat-forming legume for soil protection that has been tested by the Soil Conservation Service in Puerto Rico. Tropical kudzu, commonly grown in the Federated Malay States, was first grown by the Soil Conservation Service in Puerto Rico in 1940. Plantings have been established from seed on many types of well-drained soils where the annual rainfall is 50 inches or more. It can be established on clay subsoils where erosion has been severe or on excavated soils when the seedbed is well prepared by plowing and working in manure or chemical fertilizer. Well-established plants have extensive surface roots, and deep taproots which may extend 4 feet or more into heavy clay soils. It is through these deep roots that the plants obtain moisture during the 4- to 5-month dry season common in most sections of Puerto Rico. The plants produce about 150 pounds or more per acre of clean seed over a 4- to 5-month period beginning about November. Good germination may be expected in 10 days from seed which has been treated with a 50-percent solution of sulfuric acid for 30 minutes, washed, and dried.

The chemical analyses [17] are shown in table 10 for three samples of tropical kudzu.

Table 10.—*Analyses of tropical kudzu from the station planting in Mayaguez*

Element	Dates			Average
	May 1944	January 1945	April 1945	
Ash [1]	5.84	5.85	8.00	6.56
Proteins	15.38	14.95	22.78	17.70
Ether extract	3.64	3.91	4.84	4.13
Fiber	33.29	34.33	28.62	32.08
Nitrogen free extract	41.85	40.96	35.76	39.53

[1] The average percentage of moisture in green forage was 75 percent. All figures expressed on dry basis.

Tropical kudzu has produced from 12 to 20 tons of green forage per acre per year depending on soil fertility and climatic conditions. It has been grazed with success by work oxen, milk cows, and goats. This legume will spread under natural conditions when seeding is permitted, but it can be easily destroyed by plowing. The indications at this time are that it will not become a pest on cultivated lands.

The Federal Experiment Station in cooperation with the Soil Conservation Service improved about 7 acres of station grass pasture by establishing 4 acres in molasses grass (*Melinis minutiflora* Beauv.) and 3 acres in trailing indigo (*Indigofera endecaphylla* Jacq.). The purpose of this planting was to determine yields per acre and if trailing

[16] United States Soil Conservation Service.

[17] Analyses by Víctor L. Quiñones, assistant chemist, Agricultural Experiment Station of the University of Puerto Rico.

indigo had any toxic effect on milk cows. Each acre received 2 tons of limestone screenings and 600 pounds of a 6–9–10 chemical fertilizer. The complete cost of establishing the molasses grass per acre by planting seed was $35 against $57 for trailing indigo planted from cuttings. More labor for weeding was required to establish the trailing indigo than the molasses grass. Replicated plots of the two classes of forage have been cut, and weights taken for the first 6 months of 1945. During this period the molasses grass produced 7 tons of green forage per acre and the trailing indigo 6 tons. Six milk cows have grazed on these plots continuously for the past 4 months and their weight, milk production, and general appearance have improved. No toxic effects from trailing indigo have been observed.

PUBLICATIONS

DISTRIBUTION OF REPORTS

Requests increased for Spanish edition of annual report.—Quarterly reports summarizing the current work of the station were mimeographed as usual for interoffice circulation and for distribution on request to those professionally interested in the subjects covered. The mailing list contained 72 names, 12 of which were in 7 foreign countries.

The annual report for the fiscal year 1943, 38 pages in length, was issued in July 1944, 333 copies being sent on request or exchange to individuals and institutions in the United States and possessions and 139 to 23 foreign countries. The Spanish translation of this report was issued in August 1944 and similarly circulated among 343 addressees in in Puerto Rico and 86 in 13 Latin American countries.

PUBLICATIONS ISSUED

The following publications were issued during the year:

ARANA, FRANCISCA E. Vanilla curing and its chemistry. Puerto Rico (Mayaguez) Fed. Expt. Sta. Bul. 42, 17 pp., illus. 1944.

ARANA, FRANCISCA E. Vanilla curing. Puerto Rico (Mayaguez) Fed. Expt. Sta. Cir. 25, 21 pp., illus. 1945.

The following articles were published by the station staff in periodicals outside the Department:

ARANA, FRANCISCA E., and KEVORKIAN, A. G. Relation of moisture content to quality of vanilla beans. Puerto Rico Univ. Jour. Agr. 27: 105–116, illus. 1943.

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